Ocular Dominance Plasticity Under Metabotropic Glutamate Receptor Blockade

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Science, New Series, Vol. 272, No. 5261 (Apr. 26, 1996), 554-557.

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tinued to decay very slowly (compare middle panels of Fig. 3, A and C). This large mismatch between oxygen consumption and supply probably underscores the importance of adequate oxygen supply: watering the entire garden for the sake of one thirsty flower (*21*).

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11. Five cats were anesthetized with continuous infusion of sodium pentothal after an initial induction using ketamine HCl and atropine sulphate. All surgical and experimental procedures were in accordance with NIH guidelines. A craniotomy was performed, overlying area 18 of the visual cortex. A stainless steel chamber was mounted over the craniotomy. Each stimulus was presented 24 to 128 times, in randomized order. The intrinsic optical signals were imaged by a slow-scan CCD (charge-coupled device) camera (*6*) or enhanced video system (Imager 2001; Optical Imaging, Germantown) attached to a dual macroscope (*22*).
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13. To calculate the mapping spectra, we first computed the global signal spectrum for every cortical location in images obtained during vertical-grating and horizontal-grating stimulation. Each spatiospectral mapping image was then calculated as a difference between the above images. Each average mapping spectrum (Fig. 2H) was calculated as the difference between the average spectra from regions that were maximally activated by the vertical grating and the average spectra from regions maximally activated by the horizontal grating.
14. Spectral decomposition: For small changes, each experimental spectrum can be expressed as

$$\Delta OD_\lambda = K_1\varepsilon(HbO_2) + K_2\varepsilon(Hbr) + LS$$

where K_1 is related to the product of increased HbO_2 tissue concentration and the optical path length; similarly, K_2 is the corresponding parameter for Hbr. The textbook extinction coefficients $\varepsilon(HbO_2)$ and $\varepsilon(Hbr)$ were used. A least squares routine was used to fit the experimental data (root mean square of error was <5%). LS was assumed constant. Using other wavelength-dependent models for the LS contribution did not significantly affect the values of K_1 and K_2. Modeling the contribution from cytochromes proved that at the wavelength range we used, their contribution to the observed spectral changes was minimal.
15. Each mapping component was calculated as a difference between the corresponding global components obtained during vertical and horizontal grating stimulation. We then calculated the difference in the average intensity in regions that were maximally selective for either stimulus.
16. A distinction between the various components of light scattering has not been made here. The LS signal is useful for functional optical imaging, whenever large vascular noise is associated with an experiment. High-quality functional maps were obtained at a wavelength longer than 660 nm, at which the contribution of light scattering to the mapping components was larger than 70%.
17. Both f-MRI and PET studies were performed on awake human subjects, whereas the present experiments were performed on anesthetized cats. However, we observed a similar "initial dip" in experiments performed on awake monkeys.
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23. We thank U. Dirnagl, A. Villringer, I. Steinberg, A. Artsen, and R. Malach for their useful comments. We also thank I. Silver, P. Jezzard, B. Chance, and R. Turner for their helpful contribution. Special thanks to A. Ariel, D. Glaser, D. Shoham, A. Shmuel, and I. Vanzetta for their help during experiments and to N. Dekel, D. Ettner, C. Wijnbergen, and S. Leytus for their technical help.

7 December 1995; accepted 23 February 1996

Ocular Dominance Plasticity Under Metabotropic Glutamate Receptor Blockade

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Occluding vision through one eye during a critical period in early life nearly abolishes responses to that eye in visual cortex. This phenomenon is mimicked by long-term depression of synaptic transmission in vitro, which may require metabotropic glutamate receptors (mGluRs) and is age-dependent. Peaks in mGluR expression and glutamate-stimulated phosphoinositide turnover during visual cortical development have been proposed as biochemical bases for the critical period. Pharmacological blockade of mGluRs specifically prevented synapse weakening in mouse visual cortical slices but did not alter kitten ocular dominance plasticity in vivo. Thus, a heightened mGluR response does not account for the critical period in development.

Connections in the developing vertebrate visual system are sculpted by an activity-dependent competition between inputs for common postsynaptic neurons. Manipulations of visual experience, such as monocular deprivation (MD) during a well-defined critical period, regulate cortical physiology and ultimately lead to anatomical rearrangements (*1*). The biochemical basis for experience-dependent changes in visual circuitry remains largely unknown. mGluRs are reported to play a role in the neural plasticity of several systems, including synapse strengthening in the hippocampus (*2–4*) and long-term depression (LTD), a form of age-dependent (*5*) synapse weakening in the hippocampus (*6*), neocortex (*7*), and cerebellum (*8*). Expression of mGluRs (*9*) and glutamate-stimulated phosphoinositide (PI) turnover (*10*) have both been shown to peak transiently during development of cat primary visual cortex, concurrent with the height of sensitivity to visual deprivation. Thus, mGluR function is a candidate mediator of cortical plasticity, accounting for both the time course of the critical period and the loss of responsiveness from an eye deprived of vision. We have now examined developmental plasticity of primary visual cortex both in vitro and in vivo with the mGluR antagonist α-methyl-4-carboxyphenylglycine (MCPG).

The mechanisms responsible for the formation of ocular dominance columns during normal development are thought to underlie the effects of MD (*1*). At the peak of the critical period in the cat (4 weeks after birth), significant segregation of the afferent axons serving the two eyes has already taken place (*11*). Thus, we examined depotentiation of experimentally potentiated responses, rather than depression of naïve synapses, as the most appropriate in vitro model for the loss of responses through the deprived eye. Theta-burst stimulation (TBS), to produce

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long-term potentiation (LTP), and low-frequency stimulation (1 Hz), to elicit depression of transmission from layer IV to II/III in visual cortex in vitro, have been advocated as physiologically relevant models for understanding naturally occurring synapse modifications (*12*). Visual experience similarly regulates functional development of visual cortex across mammalian species (*1*, *13*), and slices of mouse visual cortex also exhibited a TBS-induced LTP that could be depotentiated by 1-Hz stimulation (Fig. 1C) (*14*).

Specific blockade of mGluR activation and depotentiation by MCPG was confirmed in developing mouse visual cortex in vitro. Activation of postsynaptic PI-linked mGluRs by the specific agonist 1*S*,3*R*-1-aminocyclopentane-1,3-dicarboxylate (t-ACPD) depolarizes neurons by closing potassium channels, such as the calcium-activated after-hyperpolarizing potassium current (I_{AHP}) (*15*). MCPG prevented the t-ACPD effect on I_{AHP} in visual cortical pyramidal cells (Fig. 1A) (*16*). Bath application at least 10 min before and throughout 1-Hz stimulation also reversibly prevented depotentiation in vitro (Fig. 1, B and C). Instead, a robust and persistent potentiation was unmasked, unlike the depotentiation produced in control solutions. In contrast, TBS-induced LTP was not affected by MCPG in visual cortical slices (Fig. 1C). Potentiation under mGluR blockade after a stimulus that would normally induce LTD has also been observed in the hippocampus (*6*) and cerebellum (*8*).

Having demonstrated an essential role for mGluRs in synaptic weakening in vitro, we examined whether mGluR activation was necessary for the loss of functional inputs in vivo resulting from MD. Stereotaxically implanted cannulae delivered vehicle or MCPG solutions from osmotic minipumps to kitten striate cortex for 1 week, including 5 days of MD at the peak of the critical period (*17*). When extracellular unit recordings were made in area 17 at the end of the week blind to the minipump contents, the ocular dominance of neuronal responses was strongly shifted in favor of the open eye (Fig. 2A). The loss of deprived-eye responses was indistinguishable across hemispheres treated with vehicle or with inactive or active MCPG solutions (Table 1).

To confirm the efficacy of drug treatment in vivo, we lowered iontophoretic electrodes into regions of cortex where ocular dominance had previously been mapped and shown to have shifted (*18*). Control visual responses were vigorous even within 50 μm of the alkaline vehicle source, and cells were activated by similar iontophoretic currents for both the ionotropic glutamate receptor agonist kainic acid and the mGluR-specific agonist t-ACPD (Fig. 2C, left). In contrast, neurons in the MCPG-treated hemispheres were rarely depolarized to spike threshold by

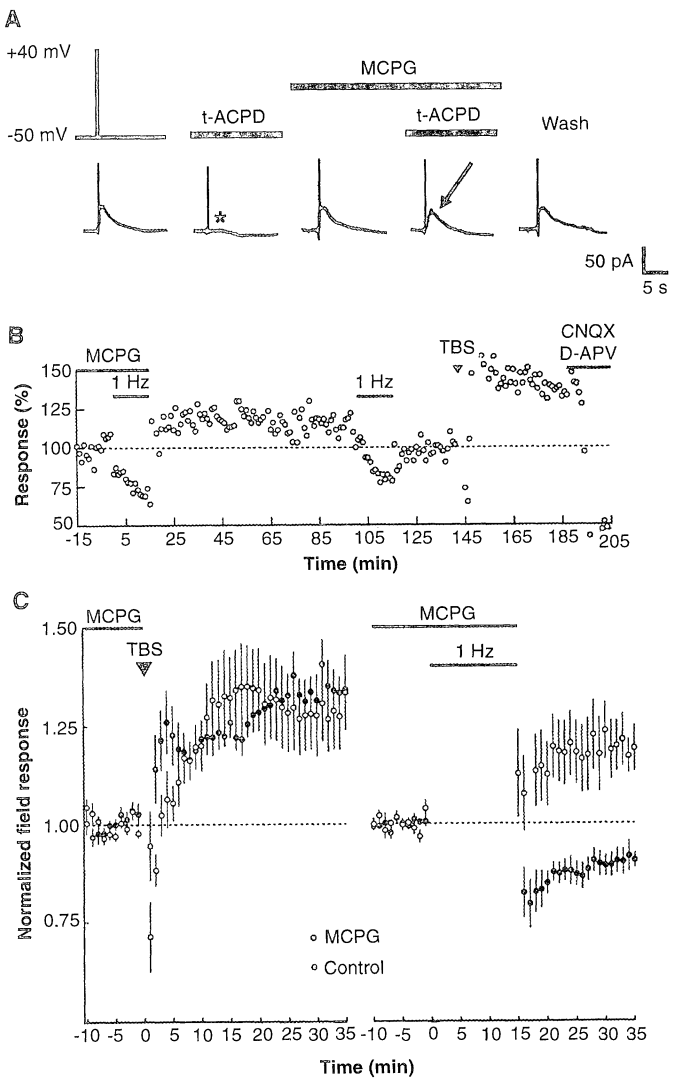

Fig. 1. Synaptic depotentiation in primary visual cortex mediated by metabotropic glutamate receptors. (**A**) Activation of postsynaptic mGluRs by the specific agonist t-ACPD (20 μM) blocked the I_{AHP} potassium current (*), which is normally opened by calcium influx through voltage-gated calcium channels in response to membrane depolarization. Bath application of MCPG (500 μM) had no effect on I_{AHP} itself, but prevented abolition of the potassium current by t-ACPD (arrow). (**B**) When MCPG was present for at least 10 min before and during 1-Hz stimulation (bar), previously potentiated synaptic responses failed to depotentiate, but were clearly capable of depression and repotentiation once the drug had washed out. Baseline field potentials were first potentiated by TBS (not shown) and their synaptic component determined at the end of the experiment by ionotropic receptor antagonists (CNQX and D-APV). (**C**) Ensemble averages of synaptic field potential slope measurements revealed that depotentiation was specifically prevented by mGluR blockade. TBS (arrowhead) induced LTP of similar magnitude in control (●) and 500 μM MCPG (○) solutions (20 min post-TBS = +29 ± 3% versus +33 ± 10%, n = 9 and 8, respectively; $P > 0.7$, Student's t test). In contrast, subsequent 1-Hz stimulation (bar) depotentiated control responses, but unmasked a further persistent potentiation when delivered in the presence of MCPG (−10 ± 3% versus +19 ± 6%, respectively, 20 min post–1 Hz; $P < 0.002$, Student's t test).

Table 1. Monocular deprivation effects in the presence of MCPG. MD induced a marked loss of responsiveness from the deprived eye regardless of whether the hemisphere was infused with vehicle or with inactive or active MCPG solution ($P > 0.3$, Mann-Whitney U test). CBI denotes contralateral bias index, where values of 1.00 and 0.00 represent complete dominance by the closed or open eye, respectively (*17*).

Animal	MCPG hemisphere			Vehicle hemisphere	
	Isomer	No. of cells	CBI	No. of cells	CBI
Exp 1	*R,S*	64	0.26	15	0.09
Exp 2	*R,S*	24	0.10	22	0.03
Exp 3	*R,S*	36	0.08	31	0.15
Exp 4	(+)	42	0.03	40	0.12
Total active		171	0.11		
Con 1	Inactive	41	0.09	16	0.09
Con 2	Inactive	19	0.10	14	0.18
Con 3	Inactive	33	0.07	36	0.28
Total inactive		93	0.09		
Total vehicle				174	0.15

t-ACPD, although they were strongly driven by visual stimulation and control-level kainic acid ejection currents (Fig. 2C, right). Grouped data demonstrated a greater than fivefold increase in activation threshold specifically for t-ACPD as compared with control units recorded in the opposite hemisphere or more distant in the same hemisphere (Fig. 2B). Consistent with acute iontophoresis results in the cerebellum, somatosensory cortex, and thalamus of rats (*19*), chronic MCPG infusion in vivo selectively blocked postsynaptic effects of mGluRs in kitten striate cortex without altering visual responsiveness.

A reduction by about 50% in the size of deprived-eye geniculocortical arbors accounts for much of the effect of 1-week MD (*20*). However, anatomical effects of 4 days of MD are less pronounced (*21*), and varying degrees of plasticity in striate cortex have been observed for much shorter periods of deprivation (*22*). Rapid effects of MD, which may reflect an important transitional state when deprived-eye afferents are anatomically present but functionally ineffective, also did not require mGluR activation. Ocular dominance plasticity produced by 2 days of MD was similar in magnitude after 50 mM MCPG infusion [contralateral bias index (CBI) = 0.12, n = 51 cells] to control (CBI = 0.10, n = 284 cells).

Thus, postsynaptic mGluR blockade did not impede ocular dominance plasticity in primary visual cortex. Molecular cloning has revealed at least eight different subtypes of mGluRs coupled to GTP-binding proteins, which can be classified into three subgroups (*23*). Of particular relevance is the finding that MCPG blocks postsynaptic group I (mGluR 1 and 5)–mediated inositol trisphosphate formation (*24*, *25*) and subsequent calcium release from internal stores (*4*, *26*). Both racemic *R,S*-MCPG and the stereoselective isomer (+)-MCPG failed to block plasticity in vivo (Table 1), indicating that none of the known PI-linked mGluR splice variants subserves the developmental critical period (*27*). Moreover, MCPG at the concentrations used in vivo is reported to be a broad-spectrum antagonist of mGluR subtypes, including postsynaptic group II (mGluR 2 and 3) (*24*) and presynaptic group III (mGluR 6, 7, and 8) receptor coupling to the cyclic AMP signaling pathway (*28*).

Certain forms of potentiation and depression of synaptic transmission in vitro in hippocampus and neocortex have been proposed to underlie activity-dependent changes in the intact animal (*12*). Our results extend earlier findings that mGluRs specifically mediate LTD of naïve synapses in rat visual cortex (*7*), with no role in TBS-induced LTP, and suggest that neocortical depotentiation and LTD share similar mechanisms. Our data are also in agreement with evidence (*29*) against mGluR involvement in hippocampal LTP induction (*3*, *4*). An mGluR-mediated molecular "switch" of naïve or depotentiated synapses to an mGluR-independent state has been proposed to explain the discrepant findings (*30*). Our results show that experience-dependent changes in visual cortical circuitry do not pass through such mGluR-sensitive naïve states. Developmental plasticity in cortex in vivo may differ from hippocampal plasticity in vitro.

The electrophysiological changes in neuronal excitability assayed here are consistent with a blockade of at least the postsynaptic PI-coupled mGluRs (*4*, *15*). Indeed, greater than 1 mM MCPG effectively antagonizes t-ACPD–induced PI turnover and subsequent plasticity in the hippocampus in vivo (*3*). Nevertheless, ocular dominance plasticity after MD in the presence of MCPG was normal. Thus, the peaks in mGluR expression (*9*) and receptor-mediated PI turnover (*10*) at the height of the critical period do not underlie the activity-dependent refinement of connections in primary visual cortex.

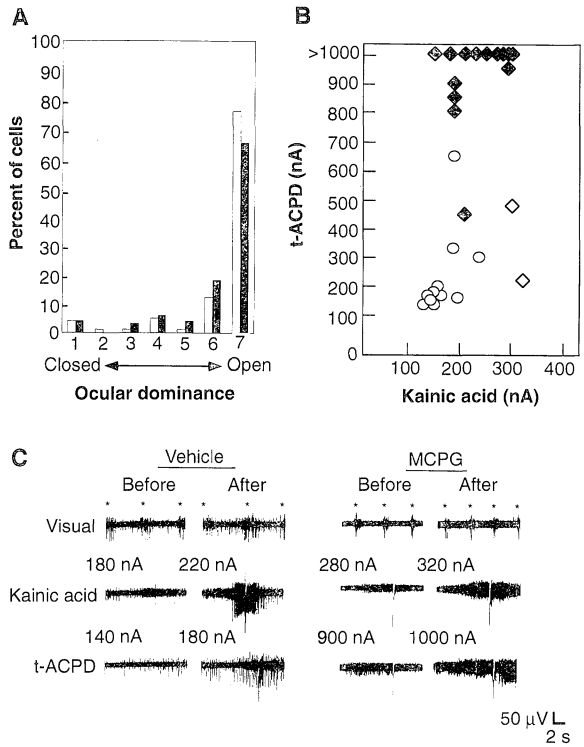

Fig. 2. Loss of deprived-eye responses after monocular deprivation during metabotropic glutamate receptor blockade. (**A**) Ocular dominance of neuronal responses shifted markedly in favor of the open eye in both hemispheres of each animal infused with vehicle (open bars) or active MCPG (solid bars) solutions (CBI = 0.14 versus 0.11; n = 108 and 171 cells, respectively, in four kittens; $P > 0.7$, Student's t test) (*17*). (**B**) Selective antagonism of metabotropic glutamate receptors by MCPG in kitten visual cortex in vivo. Control units either in vehicle-treated cortex (○) or in the same hemisphere distant from the MCPG infusion site (◇) had similar t-ACPD thresholds. Neurons in the presence of MCPG (◆) were almost never activated by t-ACPD, despite normally low kainic acid thresholds, even when ejecting currents reached the limits of the iontophoresis (1000 nA). (**C**) The ionotropic glutamate receptor agonist kainic acid or the mGluR-specific agonist t-ACPD were alternately iontophoresed onto cells isolated by visual stimulation (*) in regions of cortex where ocular dominance had previously been mapped (*18*). Activation thresholds for each drug were arbitrarily determined as the minimum ejecting current required to attain maximal cell firing within a 10-s application period (note pulse offset artifacts). Kainic acid evoked brisk spikes in both vehicle- and MCPG-treated hemispheres with low threshold currents. Ejecting t-ACPD from a neighboring barrel at similar currents depolarized control units to spike threshold. Although visually driven, neurons in MCPG-treated cortex typically did not exhibit a response to the metabotropic agonist. The example illustrates rare activation of a large unit just at the offset of a 1000-nA t-ACPD pulse. Visual responsiveness of individual cells remained robust even after strong iontophoretic currents.

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14. Recordings were obtained from layer II/III in the binocular zone of 400-μm-thick coronal slices of mouse primary visual cortex (C57/BL6; <6 weeks old) continuously superfused with oxygenated (95% O_2, 5% CO_2) artificial cerebrospinal fluid (ACSF), containing (in millimolar) 119 NaCl, 2.5 KCl, 1.3 $MgSO_4$, 1.0 NaH_2PO_4, 26.2 $NaHCO_3$, 2.5 $CaCl_2$, and 11 glucose. Extracellular pipettes (1 M NaCl, 1-3 megohm) monitored stable, half-maximal baseline field potentials evoked from layer IV by a bipolar Pt-Ir electrode delivering 100-μs pulses at 0.1 Hz. Five episodes of TBS were given at 10-s intervals to induce LTP before depotentiation was attempted with 900 pulses at 1 Hz. A single TBS consisted of 10 repetitions of four stimuli at 100 Hz delivered at 200-ms intervals (*12*). All experiments were terminated by bath application of 10 μM 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX, Tocris, UK) and 50 μM D-(−)-2-amino-5-phosphonovaleric acid (D-APV, Sigma) to determine the synaptic component of the field response. Similar results were obtained by measurements of synaptic slope or peak amplitude normalized to the baseline period before TBS, and renormalized to the 10 min preceding 1-Hz stimulation to adjust for variable elapsed time post-TBS across experiments. Both LTP and depotentiation were prevented by the *N*-methyl-D-aspartate receptor antagonist APV, as also reported for LTD of naïve synapses (*12*). *R,S*-α-MCPG or (+)-MCPG (Tocris) were dissolved in 100 mM NaOH at 50 mM, then diluted to 500 μM in ACSF.
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16. In visual cortical slices prepared and maintained as described (*14*), I_{AHP} currents were evoked by stepping the membrane potential of regular-spiking supragranular pyramidal cells [D. A. McCormick *et al.*, *J. Neurophysiol.* **54**, 782 (1985)] from −50 to +40 mV for 100 ms in the whole-cell voltage-clamp mode (Axoclamp-2B). The pipette solution contained (in millimolar): 122.5 potassium gluconate, 17.5 KCl, 10 Hepes buffer, 0.2 EGTA, 8 NaCl, 2.0 Mg–adenosine 5′-triphosphate, and 0.3 Na_3-guanosine 5′-triphosphate (3 to 8 megohm, pH 7.2, 290 to 300 mOsm). t-ACPD (Tocris) was dissolved in ACSF and bath applied.
17. In vivo experiments were done as described [H. O. Reiter, D. M. Waitzman, M. P. Stryker, *Exp. Brain Res.* **65**, 182 (1986)]. Infusion cannulae connected to osmotic minipumps (Alza model 2002) were implanted bilaterally into postnatal day 28 (P28) kitten striate cortex under sterile conditions. One eyelid was sutured shut under brief halothane anesthesia on P30, and MD was verified for 5 days. *R,S*-α- or (+)-MCPG (25 to 50 mM in 100 mM NaOH), or vehicle (100 mM NaOH, pH 10) solution was delivered at a constant rate (0.5 μl/hour) throughout the experiment. In some cases, MCPG solutions were first neutralized to physiological pH 7, which rendered the drug inactive on the in vitro I_{AHP} assay (*16*). On P35, animals were prepared for acute single-unit recording by standard techniques in accordance with University of California, San Francisco, guidelines for animal care. In brief, kittens were anesthetized and maintained with a combination of barbiturate infusion [pentobarbital sodium (Nembutal) 10 mg/kg intravenous] and N_2O:O_2 (2:1) ventilation. Extracellular unit recordings were obtained immediately in front and no further than 1.5 mm from each cannula with resin-coated tungsten microelectrodes (1 to 3 megohms) in vertical penetrations spaced evenly at 400-μm intervals along the medial bank. Electrode tracks were reconstructed in Nissl-stained coronal sections to confirm sampling from all layers of visual cortex. Light bar stimuli were swept across the receptive field with a hand-held lamp to assign each cell to an ocular dominance group on the basis of Hubel and Wiesel's seven-point scale (*1*). Here, an ocular dominance of 7 represents complete dominance by the open eye. The contralateral bias index (CBI), a weighted average of the bias toward one eye or the other, was calculated for each treated hemisphere, separately and as a group, according to the formula: $CBI = [(n_1 - n_7) + \frac{2}{3}(n_2 - n_6) + \frac{1}{3}(n_3 - n_5) + N]/(2N)$, where N is the total number of cells and n_x is the number within ocular dominance group x.
18. In each hemisphere, multibarreled iontophoretic pipettes were lowered into striate cortex just beyond the most distant penetration site used for determination of ocular dominance (<2 mm from cannula). Activation thresholds for kainic acid and t-ACPD (both 20 mM in saline) were determined by gradually increasing iontophoretic ejection currents (WPI model 160) in 20-nA steps once every 60 s. Each round of iontophoresis was preceded by isolation of multiple units with visual stimulation and concluded by verifying the presence of the same visually driven cells. Additional iontophoretic penetrations amidst the single-unit sites and well beyond (>3 mm) confirmed that all shifted cells lay within a region in which mGluRs were blocked.
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31. We thank S. Harris for assistance during the implant surgeries, and M. Fagiolini and R. A. Nicoll for critical comments on the manuscript. T.K.H. is a Howard Hughes Medical Institute Predoctoral Fellow. Supported by grants to M.P.S. from the National Institutes of Health (EY02874) and the Human Frontiers Science Program (RG69/93).

3 October 1995; accepted 30 January 1996

Transcription-Coupled Repair Deficiency and Mutations in Human Mismatch Repair Genes

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Deficiencies in mismatch repair have been linked to a common cancer predisposition syndrome in humans, hereditary nonpolyposis colorectal cancer (HNPCC), and a subset of sporadic cancers. Here, several mismatch repair–deficient tumor cell lines and HNPCC-derived lymphoblastoid cell lines were found to be deficient in an additional DNA repair process termed transcription-coupled repair (TCR). The TCR defect was corrected in a mutant cell line whose mismatch repair deficiency had been corrected by chromosome transfer. Thus, the connection between excision repair and mismatch repair previously described in *Escherichia coli* extends to humans. These results imply that deficiencies in TCR and exposure to carcinogens present in the environment may contribute to the etiology of tumors associated with genetic defects in mismatch repair.

Nucleotide excision repair helps cells tolerate exposure to various DNA-damaging agents present in the environment by removing helix-distorting lesions from cellular genomes. The general strategy appears to be similar in organisms ranging from *Escherichia coli* to humans. This process is complex and requires the participation of a number of different proteins (*1*). Its role in ameliorating the carcinogenic consequences of DNA damage has been inferred from studies of the genetic disease xeroderma pigmentosum (XP). Cells from XP patients are hypersensitive to the killing and mutagenic effects of ultraviolet light (UV) and

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