There are numerous reports of melatonin rhythmically synthesized by cultured pineal glands of nonmammalian vertebrates (*1*), and we have recently detected rhythmic melatonin synthesis in the cultured retinas and parietal eyes of the lizard *Iguana iguana* and in cultured retinas of lamprey (*Petromyzon marinus*) (9). Taken together, these observations suggest that all organized vertebrate photoreceptive structures synthesize melatonin under the control of circadian oscillators. It is an obvious corollary of this hypothesis that melatonin, and more specifically, circadian rhythmicity of melatonin level, is essential for normal photoreceptor function.

REFERENCES AND NOTES

1. M. Menaker and S. Wisner, *Proc. Natl. Acad. Sci. U.S.A.* **80**, 6119 (1983); J. S. Takahashi, N. Murakami, S. S. Nikaido, B. L. Pratt, L. M. Robertson, *Recent Progr. Horm. Res.* **45**, 279 (1989); J. Falcon and J. P. Collins, *Cell Tissue Res.* **265**, 601 (1991).
2. G. M. Cahill and J. C. Besharse, *Prog. Retinal Eye Res.* **14**, 267 (1995).
3. J. S. Takahashi and M. Menaker, *J. Neurosci.* **2**, 815 (1982); D. S. Janik, G. E. Pickard, M. Menaker, *J. Comp. Physiol. A* **166**, 811 (1990); S. Ooka-Souda, T. Kadota, H. Kabasawa, *Neurosci. Lett.* **164**, 33 (1993).
4. D. K. Welsh, D. E. Logothetis, M. Meister, S. M. Reppert, *Neuron* **14**, 697 (1995).
5. M. Terman and J. Terman, *Ann. N.Y. Acad. Sci.* **453**, 147 (1985); C. E. Reme, A. Wirz-Justice, M. Terman, *J. Biol. Rhythms* **6**, 5 (1991); J. S. Terman, C. E. Reme, M. Terman, *Brain Res.* **605**, 256 (1993).
6. G. M. Cahill and J. C. Besharse, *J. Neurosci.* **11**, 2959 (1991).
7. We used a modification of the method of M. Rollag and G. Niswender [*Endocrinology* **98**, 482 (1976)]. The lower limits of the assay were 1.8 to 2.5 pg per tube, and the upper limits were 500 pg per tube.
8. M. R. Ralph and M. Menaker, *Science* **241**, 1225 (1988); M. R. Ralph, R. G. Foster, F. C. Davis, M. Menaker, *ibid.* **247**, 975 (1990).
9. M. Menaker and G. Tosini, in *Sixth Sapporo Symposium on Biological Rhythm*, T. Hiroshige and K. Honma, Eds. (Hokkaido Univ. Press, Sapporo, Japan, in press).
10. Analysis of the time-series data was performed by an iterative, coupled Fourier transform–nonlinear least squares estimation procedure developed at the National Science Foundation Center for Biological Timing at the University of Virginia [M. L. Johnson and S. G. Frasier, *Methods Enzymol.* **117**, 301 (1985); M. Straume, S. G. Frasier-Cadoret, M. L. Johnson, in *Topics in Fluorescence Spectroscopy: Principles*, J. R. Lakowicz, Ed. (Plenum, New York, 1991), vol. 2, p. 177]. This software is available from the National Science Foundation Center for Biological Timing at the University of Virginia.
11. A fiber optic light powered by a 150-W tungsten quartz-halogen lamp was used in the experiments in which the cultured retinas were exposed to L:D cycles. The light source was located outside the incubator, and the tip of the fiber-optic probe was inside the incubator about 15 cm from the plate containing the cultures. We estimated light intensities at the level of the retinas by placing an irradiance detector of a UDT 350 radiometer-photometer (United Detector Technology, Hawthorne, CA) at the same location as the superfusion chamber.

12. Supported by International Human Frontier Science Program fellowship (LT-145/93) to G.T. and NIH grant (HD-13162) to M.M. We thank M. Straume for the statistical analysis, G. Cahill for discussion, and M. Rollag for the melatonin antibody.

2 November 1995; accepted 8 February 1996

Requirement for α-CaMKII in Experience-Dependent Plasticity of the Barrel Cortex

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The mammalian sensory neocortex exhibits experience-dependent plasticity such that neurons modify their response properties according to changes in sensory experience. The synaptic plasticity mechanism of long-term potentiation requiring calcium-calmodulin–dependent kinase type II (CaMKII) could underlie experience-dependent plasticity. Plasticity in adult mice can be induced by changes in the patterns of tactile input to the barrel cortex. This response is strongly depressed in adult mice that lack the gene encoding α-CaMKII, although adolescent animals are unaffected. Thus, α-CaMKII is necessary either for the induction or for the expression of plasticity in adult mice.

In mammals, the somatosensory cortex contains a complete map of the body surface. The map is capable of undergoing functional reorganization in response to manipulations of the periphery (*1*, *2*). In rodents, experience of the world through a single whisker, rather than the normal array of five rows of whiskers, results in increased representation of the spared vibrissa in the cortex (*3*, *4*). Vibrissa deprivation plasticity has a number of properties in common with ocular dominance plasticity in the visual cortex, including a critical period for plasticity within cortical layer IV and a longer period for plasticity in layers II and III (*4*, *5*).

The molecular mechanisms that underlie experience-dependent plasticity remain unclear. Plasticity could be achieved if a molecular switch, such as an autophosphorylating kinase, were located at the synapse (*6*, *7*). The abundant postsynaptic protein calcium-calmodulin–dependent kinase type II (CaMKII) has the appropriate properties (*7*, *8*). Indeed, some forms of synaptic plasticity, such as long-term potentiation (LTP) in the hippocampus, are dependent on

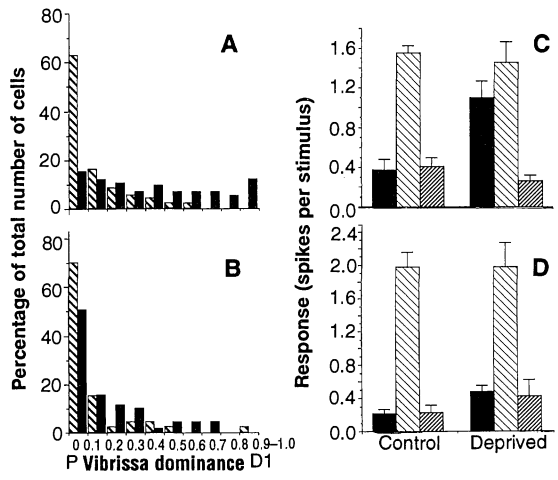

Fig. 1. Plasticity in normal adult mouse neocortex induced by vibrissa deprivation. (**A** and **B**) Pooled data for cells recorded in barrel columns immediately surrounding D1. In undeprived mice (hatched bars, 95 neurons) the VDH (*16*) was biased toward the principal vibrissa (P), and most cells showed no response to the D1 vibrissa. After deprivation (black bars, 118 neurons), the distribution shifted toward the spared vibrissa (D1) in layers II and III (A), such that 32% of neurons showed greater responses to D1 than to the principal vibrissa. The effect was less pronounced in layer IV (B), where 50% still showed no response to D1. The WVDI estimates variations in VDHs between animals (*16*) and changed significantly for cells in layers II and III after deprivation (A) (control mean ± SD = 0.11 ± 0.09; deprived mean = 0.51 ± 0.19, $P < 0.05$, *t* test) but not for cells in layer IV (B) (control mean = 0.10 ± 0.09; deprived mean = 0.17 ± 0.14, $P > 0.1$, *t* test). (**C** and **D**) The absolute response magnitude is plotted for three vibrissa types: the spared D1 vibrissa (solid bars), deprived principal vibrissa (wide hatch marks), and deprived surround receptive field vibrissa (narrow hatch marks) (*16*). In layers II and III (C), the average D1 response magnitude increased almost threefold with deprivation (control mean ± SD = 0.37 ± 0.29 spikes per stimulus; deprived mean = 1.1 ± 0.47) [ANOVA, $F(1,14) = 6.5$, $P = 0.024$]. The average principal vibrissa response did not change significantly (control = 1.57 ± 0.19; deprived = 1.46 ± 0.58) [$F(1,14) = 0.80$, $P = 0.38$] nor did responses of surround vibrissae (control = 0.41 ± 0.23 and deprived = 0.26 ± 0.17) [$F(1,13) = 2.5$, $P = 0.14$]. In layer IV (D), deprivation did not cause a change in the principal vibrissa response (control mean = 1.98 ± 0.49 spikes per stimulus; deprived mean = 1.98 ± 0.77). But there was a small but significant increase in the D1 vibrissa response (control mean ± SD = 0.22 ± 0.15; deprived mean = 0.48 ± 0.20) [$F(1,13) = 8.4$, $P = 0.013$], although no significant change in the surround deprived vibrissa response (control mean = 0.23 ± 0.23; deprived mean = 0.42 ± 0.54) [$F(1,11) = 0.55$, $P = 0.47$]. The histograms show means and standard errors calculated across animals.

α-CaMKII (9). In many cortical areas, layers II and III show the highest levels of α-CaMKII immunoreactivity (*10*) as well as the most experience-dependent plasticity and the most readily evoked cortical LTP (*3, 5, 11, 12*). Sensory deprivation can also affect expression of α-CaMKII (*13*).

To test whether α-CaMKII is necessary for experience-dependent plasticity, we removed vibrissae from mice lacking the gene encoding α-CaMKII and analyzed potentiation and depression of cortical vibrissa responses (*14*). Experimenters were blind to the genotype of the animals and to the location of the recording electrode within the barrel map. All data collection was automatic and quantitative, and a standard electromechanical stimulus and poststimulus time histogram analysis were used (*15*). Three main measures were used to quantify plasticity: (i) a shift in the vibrissa dominance histogram (VDH), (ii) a change in the average response magnitude for spared and deprived vibrissae in deprived barrel columns, and (iii) an expansion of the area of cortex expressing high response levels to spared vibrissa stimulation (*16*).

Adult (older than 6 months) wild-type mice (n = 7 deprived, 7 undeprived) showed substantial plasticity. Deprivation shifted the VDH in layers II and III but not in layer IV, potentiated the spared vibrissa response, but did not depress the deprived vibrissa response (Fig. 1). Deprivation led to an expansion of the area of cortex responding at high levels to spared vibrissa stimulation (Fig. 2, A and B).

In contrast, homozygotes with both α-CaMKII genes deleted (n = 5 deprived, 2 undeprived) showed impaired plasticity. Deprivation did not shift significantly the VDH [mean weighted vibrissa dominance index (WVDI) ± SD = 0.26 ± 0.15 in deprived homozygotes and 0.17 ± 0.12 in undeprived mutants (*17*), $t(12) = 1.62$, $P > 0.2$]; even in deprived mice, high-level responses to spared vibrissa stimulation were almost entirely confined to the D1 barrel column (Fig. 2D). Furthermore, the average response of the spared vibrissae did not show an increase in barrel columns surrounding the spared vibrissa's own barrel column (Fig. 3A).

Heterozygotes (n = 6 deprived, 7 undeprived) exhibited intermediate plasticity. Deprivation caused some shift in the VDH [mean WVDI ± SD = 0.35 ± 0.11 in deprived heterozygotes and 0.17 ± 0.12 in undeprived mutants, $t(13) = 2.77$, $P < 0.02$], and the D1 map expanded, although less than in wild types (Fig. 2C). However, the average D1 response in heterozygotes did not differ significantly from either the deprived surround vibrissa response or the D1 response in undeprived mutants (Fig. 3A). Finally, in common with wild types, there was no decrease in the deprived vibrissa response in either homozygotes or heterozygotes (see Fig. 3B). These results suggest that plasticity of the (spared) D1 vibrissa response in the adult cortex is dependent on α-CaMKII.

It is highly unlikely that α-CaMKII is involved in the early development of the

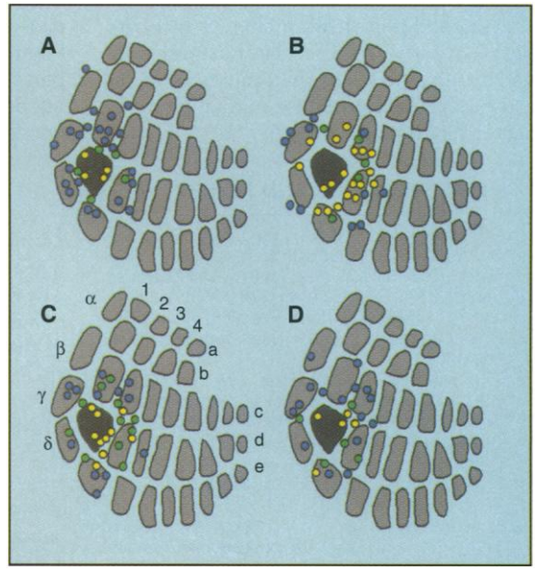

Fig. 2. Adult functional map in layers II and III of wild-type and α-CaMKII mutants. The distribution of electrode penetrations (circles) is shown relative to the layer IV barrel field (*16*). Barrel denominations are shown in (C): barrel rows a to e and arcs 1 to 4 are indicated. The caudalmost arc contains barrels α to δ. The D1 barrel is shaded darker. Color indicates average response magnitude (RM) of cells in layers II and III to D1 vibrissa stimulation (*15*) (blue, RM < 0.6 spike per stimulus; green, 0.6 ≤ RM < 1.2; yellow, 1.2 ≤ RM). In control wild types (**A**), the maximal D1 vibrissa response coincides with the D1 barrel column. In deprived wild types (**B**), the maximal D1 vibrissa response extends into neighboring barrel columns. In deprived heterozygotes (**C**), the maximal D1 vibrissa response does not extend as far. In deprived homozygotes (**D**), there is no pattern of expansion in the area of neocortex showing maximum response to the spared D1 vibrissa.

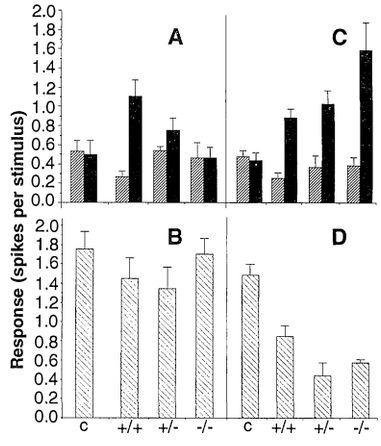

Fig. 3. Plasticity in adult (A and B) and adolescent (C and D) mice. The absolute response magnitude (*16*) is plotted in (A) and (C) for responses to the spared D1 vibrissa (black bars) and to the deprived surround vibrissae (fine hatch marks), and in (B) and (D) for responses to deprived principal vibrissa (wide hatch marks). All histograms show means and standard errors. (**A**) The D1 vibrissa responses of adults were approximately equal to surround vibrissa responses in undeprived controls (c) [ANOVA, $F(1,16) = 0.07$, $P = 0.8$]. The spared D1 vibrissa response increased after deprivation in wild types (+/+) (see legend to Fig. 1), but in heterozygotes (+/−) was not significantly different from control D1 responses [$F(1,14) = 1.63$, $P = 0.22$], nor was the response different from the surround vibrissa responses recorded from the same animals [$F(1,10) = 1.67$, $P = 0.23$]. The D1 response in homozygotes (−/−) did not increase relative to the D1 responses in controls [$F(1,13) = 0.03$, $P = 0.87$] nor did it increase relative to the surround vibrissa responses recorded in the same animals [$F(1,8) = 0.00$, $P = 0.99$]. The control group is undeprived mutants (*17*). (**B**) Responses of adults to principal vibrissa stimulation in cells outside the D1 barrel column. Principal vibrissa responses remained constant despite the deprivation and were independent of genotype (ANOVA, $P \geq 0.14$ for all cases). The lack of depression appears to be dependent on age [see (D)]. Control mutants, 128 neurons; heterozygotes, 104 neurons; homozygotes, 88 neurons. (**C**) Responses of adolescents to the spared D1 vibrissa (black bars) increased in wild types, heterozygotes, and homozygotes relative to those in undeprived (control) animals (ANOVA, $P < 0.005$ in all cases). Deprivation did not alter the deprived surround vibrissa responses (hatched bars) in heterozygotes or homozygotes (ANOVA, $P > 0.1$ in both cases) and caused a small decrease in the case of deprived wild types (ANOVA, $P < 0.05$). The control group (c) is undeprived wild types. (**D**) Responses of adolescents to the principal vibrissa were depressed by the deprivation in wild-type and mutant animals alike (ANOVA, $P < 0.005$ in all cases). Although the gene inactivation appeared to produce greater depression in heterozygotes and homozygotes than in wild types, the effect was not significant (Tukey-Kramer HSD, $\alpha = 0.05$). Controls, 87 neurons; wild-type deprived, 107; heterozygotes, 60; homozygotes, 44.

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barrel cortex (*18*). Barrels form normally in α-CaMKII–deficient mice, and receptive field properties in these mice are similar to those of wild-type mice (*19*). It is possible, however, that α-CaMKII could be involved in subsequent developmental events in the neocortex. Therefore, we analyzed plasticity in adolescent mice (1 to 2 months). Our sample included four homozygotes, four heterozygotes, and eight wild types. Potentiation of the spared vibrissa response and depression of the deprived vibrissa response occurred in the complete absence of the gene encoding α-CaMKII (Fig. 3, C and D), which implies that the lack of plasticity in adult animals cannot be explained by a developmental disruption before 1 to 2 months of age. Surprisingly, although potentiation of the D1 vibrissa response was similar in adolescent heterozygotes and wild types, it was greater than both of these in homozygotes [Tukey-Kramer honestly significant difference (HSD), $\alpha = 0.05$].

The gene encoding α-CaMKII is inactivated throughout the forebrain in the null mutants but appears to affect plasticity without affecting normal sensory processing (*19*) or hippocampal-independent behavior (*20*), arguing against a widespread or general action of the gene inactivation. Homozygotes show more limbic seizure activity than wild types (*21*) (seizures were not observed in the neocortex here). However, epilepsy does not affect cortical plasticity (*22*), and heterozygotes have depressed plasticity despite the fact that they exhibit the same levels of seizure as wild types. Differences in fear and aggression behavior between mutants and wild types (*23*) are unlikely to affect plasticity either, because they do not prevent sensory exploration. Furthermore, behavior and sensory processing appear normal enough to allow plasticity in the somatosensory (Fig. 3) and visual cortex (*24*) of adolescent homozygotes. The simplest explanation for our results is that the mutation blocks directly a potentiation process in cortical layers II and III, which could explain why α-CaMKII is normally expressed at high levels in these layers (*10*). The finding that cortical LTP is also blocked in layers II and III of adult α-CaMKII mutants (*25*) suggests that a similar synaptic mechanism may be involved in both cases. By using quantitative methods and comparing neurons in anatomically identified positions, we have been able to distinguish between potentiation and depression components of plasticity. Our results demonstrate that an experience-dependent, α-CaMKII–dependent potentiation mechanism is present in the adult mouse sensory cortex.

REFERENCES AND NOTES

1. M. M. Merzenich *et al.*, *Neuroscience* **8**, 33 (1983).
2. S. A. Clark, T. Allard, W. M. Jenkins, M. M. Merzenich, *Nature* **332**, 444 (1988); A. Mogiliner *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **90**, 3593 (1993).
3. K. Fox, *J. Neurosci.* **12**, 1286 (1992); P. J. Hand, in *Changing Concepts of the Nervous System*, A. R. Morrison and P. L. Strick, Eds. (Academic Press, New York, 1982), p. 49; M. Kossut, *Prog. Neurobiol.* **39**, 389 (1992).
4. K. Fox, *J. Neurosci.* **14**, 7665 (1994).
5. S. Glazewski and K. Fox, *J. Neurophysiol.* **74**, 1714 (1996).
6. J. E. Lisman, *Proc. Natl. Acad. Sci. U.S.A.* **82**, 3055 (1985); J. E. Lisman, *Trends Neurosci.* **17**, 406 (1994).
7. S. G. Miller and M. B. Kennedy, *Cell* **44**, 861 (1986).
8. M. B. Kennedy, M. K. Bennett, N. E. Erondu, *Proc. Natl. Acad. Sci. U.S.A.* **80**, 7357 (1983).
9. A. J. Silva, C. F. Stevens, S. Tonegawa, Y. Wang, *Science* **257**, 201 (1992); R. Malinow, H. Schulman, R. W. Tsien, *ibid.* **245**, 862 (1989); R. C. Malenka *et al.*, *Nature* **340**, 554 (1989).
10. D. L. Benson, P. J. Isackson, C. M. Gall, E. G. Jones, *Neuroscience* **46**, 825 (1992) (figure 3E).
11. Y. Komatsu, K. Fujii, J. Maeda, H. Sakaguchi, K. Toyama, *J. Neurophysiol.* **59**, 124 (1988).
12. M. Castro-Alamancos, J. P. Donoghue, B. W. Connors, *J. Neurosci.* **15**, 5324 (1995).
13. S. H. Hendry and M. B. Kennedy, *Proc. Natl. Acad. Sci. U.S.A.* **83**, 1536 (1986); R. L. Neve and M. F. Bear, *ibid.* **86**, 4781 (1989).
14. All but the D1 vibrissa were removed for a period of 18 days without follicle damage [X. Li *et al.*, *J. Comp. Neurol.* **357**, 465 (1995)]. Vibrissae were allowed to regrow 8 to 11 days before recording (by 10 to 15 mm). Adults were 6 to 18 months old; adolescents, 1 to 2 months. Age distributions for wild-type, heterozygote, and homozygote mice were indistinguishable (Tukey-Kramer HSD, $\alpha = 0.1$) because subjects were siblings.
15. Animals were anesthetized with urethane intraperitoneally (1.5 mg per gram of body weight). Fifty vibrissa deflections of 1° at 1 Hz evoked spikes from isolated units (counted in the 5- to 50-ms poststimulus time histogram interval minus spontaneous activity) [see M. Armstrong-James and K. Fox, *J. Comp. Neurol.* **263**, 265 (1987)]. Data were recorded for 842 layer II and III cells in barrel columns surrounding D1.
16. Recording locations within the cytochrome oxidase–stained barrel field [M. M. T. Wong-Riley, *Brain Res.* **171**, 11 (1979)] were identified from focal lesions made at the end of each penetration (1 μA, 10 s, dc tip negative). Layers II and III were 30 to 270 μm, the border of layers III and IV from 270 to 300 μm, and layer IV from 300 to 420 μm. For each cell, vibrissa dominance (F) = $d1/(d1 + p)$, where $d1$ is the average D1 vibrissa response and p is the average principal vibrissa response (*3*). In Fig. 1, the VDH shows the frequency distribution of F. For each animal, WVDI = $(0F0 + 1F1 + 2F2 + 3F3 + 4F4 + 5F5 + 6F6 + 7F7 + 8F8 + 9F9)/9N$, where $F0$ is the number of cells for which $0 \leq F < 0.1$, $F1$ is the number for which $0.1 \leq F < 0.2$, and N is the number of cells in the sample [see (*3*)]. For each animal, the average D1 (D1) and principal vibrissa responses (P) were calculated [see (*5*)]. We found that D1, P, and WVDI were normally distributed (Shapiro-Wilk test, $W > 0.92$, $P > 0.1$), and these quantities were averaged across each deprivation-genotype-age group for statistical analysis [analysis of variance (ANOVA) and Tukey-Kramer HSD]. The surround receptive field (SRF) vibrissa was taken as a deprived vibrissa that was neither the principal vibrissa nor D1 (for example, D3 for a cell in the D2 barrel).
17. Principal vibrissa responses for undeprived homozygotes and heterozygotes were indistinguishable (principal mean = 1.80 ± 0.50 spikes per stimulus for heterozygotes and 1.65 ± 0.52 for homozygotes) [ANOVA, $F(1,8) = 0.14$, $P = 0.72$], as were surround responses (SRF mean = 0.62 ± 0.25 for heterozygotes and 0.41 ± 0.25 for homozygotes) [$F(1,8) = 1.11$, $P = 0.33$] and were combined in a single "undeprived mutant" group.
18. Transcription of α-CaMKII is barely detectable at 4 days in rat neocortex [K. E. Burgin *et al.*, *J. Neurosci.* **10**, 1788 (1990)], whereas structural plasticity of the barrel map peaks on postnatal day 0 (P0) and is absent by P4 in mice [T. A. Woolsey and J. R. Wann, *J. Comp. Neurol.* **170**, 53 (1976)] and rats [B. L. Schlaggar, K. Fox, D. M. M. O'Leary, *Nature* **364**, 623 (1993)].
19. Average receptive field sizes and principal vibrissa response latencies were similar for undeprived wild types, heterozygotes, and homozygotes, respectively, in layers II and III (1.63 ± 0.87 vibrissae, $n = 62$ cells; 1.56 ± 0.63, $n = 76$; 1.59 ± 0.87, $n = 62$) and IV (1.38 ± 0.63, $n = 26$; 1.37 ± 0.49, $n = 27$; 1.14 ± 0.35, $n = 21$). Average latencies were also similar in layers II and III (mean ± SD = 14.8 ± 5.5; 16.1 ± 8.2; and 14.5 ± 6.4 ms) and IV (11.7 ± 5.0; 15.3 ± 8; and 11 ± 5.0 ms). Median response latencies were also similar in layers II and III [median, interquartile range = 14,7; 16,10; and 14,7.5) and IV (10,9; 14,2; and 10,8] ($P > 0.347$, Welch ANOVA and $P > 0.178$, Mann-Whitney U test). Vibrissae evoking a response ≥0.5 spike per stimulus were analyzed.
20. A. J. Silva, R. Paylor, J. M. Wehner, S. Tonegawa, *Science* **257**, 206 (1992).
21. L. S. Butler *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **92**, 6852 (1995).
22. T. O. Videen, N. W. Daw, R. E. Collins, *Brain Res.* **371**, 1 (1986).
23. C. Chen, D. G. Rainnie, R. W. Greene, S. Tonegawa, *Science* **266**, 291 (1994).
24. J. A. Gordon *et al.*, *Soc. Neurosci. Abstr.* **20**, 465 (1994) (abstract).
25. A. Kirkwood, A. Silva, M. F. Bear, *ibid.*, p. 1471 (abstract).

26. All procedures were approved by the animal care committees of the University of Minnesota and Cold Spring Harbor Laboratories. We thank X. Li for technical help and P. Chapman and H. Cline for reading the manuscript. Supported by NIH grant 27759 to K.F. and grants from the Beckman, Whitehall, and Klingenstein foundations to A.S.

28 November 1995; accepted 27 February 1996