The Journal of Neuroscience, June 1, 2000, *20*(11):4206–4216

Upregulation of cAMP Response Element-Mediated Gene Expression during Experience-Dependent Plasticity in Adult Neocortex

Alison L. Barth,[1] Mervyn McKenna,[1] Stanislaw Glazewski,[1] Penelope Hill,[1] Soren Impey,[2] Daniel Storm,[2] and Kevin Fox[1]

[1]*Cardiff School of Biosciences, Cardiff University, Cardiff, CF10 3US Wales, United Kingdom, and* [2]*Department of Pharmacology, University of Washington, Seattle, Washington 98195*

Gene transcription is thought to be essential for memory consolidation and long-lasting changes in synaptic function. In particular, the signal transduction pathways that activate the transcription factor cAMP response element binding protein (CREB) have been implicated in the process of synaptic potentiation. To study the involvement of this pathway in neocortical plasticity within the barrel cortex, we have used a strain of mice carrying a *LacZ* reporter gene with six cAMP response elements (CREs) upstream of a minimal promoter. Removal of all but one facial whisker results in the expansion of the spared whisker's functional representation within somatosensory cortex. Under the same conditions of whisker deprivation, we observed a strong (eightfold compared with baseline) and highly place-specific upregulation of CRE-mediated gene transcription in layer IV of the spared whisker barrel. Reporter gene upregulation occurred rapidly after deprivation (16 hr) and was only observed under experimental conditions capable of inducing whisker response potentiation. *LacZ* expression in layer IV was accompanied by an increase in responsiveness of a subpopulation of layers II/III cells to spared whisker stimulation as determined by *in vivo* single-unit recording. Given that CREB is involved in the expression of plasticity in superficial layers (Glazewski et al., 1999), and yet CRE-mediated gene expression occurs in layer IV, it is likely that the molecular events initiating plasticity occur presynaptically to the cells that exhibit changes in their receptive field properties.

Key words: barrel cortex; somatosensory; experience-dependent plasticity; gene regulation; gene expression; CREB

Memory consolidation is thought to depend on long-lasting changes in synaptic transmission, and both processes are thought to depend on protein synthesis (for review, see Davis and Squire, 1984; Montarolo et al., 1986). Long-term potentiation (LTP) is a synaptic analog of memory that appears to require protein synthesis and mRNA transcription to last >1–2 hr in the hippocampus (Frey et al., 1988; Nguyen et al., 1994). These findings raise the question of how synaptic activity, which induces LTP, can trigger gene transcription. One possibility is that synaptic activity affects gene transcription via phosphorylation of the cAMP response element binding protein (CREB) (Dash et al., 1991; Sheng et al., 1991; Yin et al., 1995). This transcription factor can be activated by a number of signal transduction pathways, including those pathways initiated by intracellular increases in cAMP and Ca^{2+} (Montminy et al., 1990; Bito et al., 1997), and induces transcription from genes containing the cAMP response element (CRE) binding site within the promoter (Gonzalez et al., 1989).

In both vertebrates and invertebrates, a great deal of evidence has accumulated that CREB, or closely related transcription factors, may be important for synaptic plasticity and learning (for review, see Tully, 1998). For example, LTP does not last >90 min in the hippocampus of animals lacking most of the major isoforms for CREB (Bourtchuladze et al., 1994). Furthermore, activation of a CRE–*LacZ* reporter gene is observed only in the presence of stimuli that induce the late phase of LTP (>4 hr) (Impey et al., 1996). Stimuli that induce late-phase LTP also induce a persistent phosphorylation of CREB, which is thought to be necessary for transactivation of the reporter gene (Impey et al., 1996).

Little is known at present about whether gene transcription is necessary for experience-dependent plasticity. Evidence suggests that experience may be linked to CRE-mediated gene transcription, either during fear conditioning (Impey et al., 1998a) or after monocular deprivation during the critical period (Pham et al., 1999). However, a diffuse pattern of transgene activation often precludes an unambiguous correlation of the site of gene upregulation and the locus of plasticity. Occasionally, the site of plasticity will be inferred from the site of gene upregulation. To examine more closely the role of CRE-mediated gene transcription in experience-dependent plasticity therefore requires a system in which the locus of plasticity can be identified clearly and independently.

The barrel cortex is an excellent system in which to study this type of question for several reasons. First, it has been well established that potentiation of neuronal responses to whisker stimulation occur as a result of changes in sensory input (Simons and Land, 1987; Fox, 1992; Diamond et al., 1994). Second, the clearly defined anatomical map of the sensory whisker pad (Woolsey and Van der Loos, 1970) can be used to show whether changes in expression are specific to changes in experience through particular whiskers. We therefore performed a series of studies to investigate where CRE-mediated gene transcription occurred in

Received Nov. 17, 1999; revised Feb. 25, 2000; accepted March 1, 2000.

This work was supported by a Hitchings-Elion fellowship from the Burroughs Wellcome Fund (A.L.B.), National Institutes of Health Grant NS27759 (K.F.), and the Medical Research Council (K.F.). We gratefully acknowledge the assistance of Phil Blanning for animal genotyping.

Correspondence should be addressed to Kevin Fox, Cardiff School of Biosciences, Cardiff University, Cardiff, CF10 3US Wales, UK. E-mail: foxkd@cardiff.ac.uk.

Dr. Barth's present address: Department of Psychiatry, Stanford University School of Medicine, Palo Alto, CA 94304.

 0270-6474/00/204206-11$15.00/0

Table 1. The number of brains reacted and examined are shown for each stage of the process

Condition	Number of heterozygote transgenics	Cases expressing transgene	Cases sectioned and inspected	Cases quantified
D1 spared 16 hr (cortex)	40	17	12	4
D1 spared 16 hr (thalamus)			3	0
D1 spared 7 d	20	9	9	6
All deprived 16 hr	20	4	4	4
Undeprived	27	7	4	4
Totals	107	37	32	18

The left-hand column gives the total number of brains reacted from animals genotyped as transgenic for *LacZ*. Not all of these brains reacted, as shown in column 2. Overall, 35% of the cases reacted. Almost all cases that reacted were sectioned either coronally or horizontally and inspected as shown in column 3. Most but not all of these could be quantified as shown in column 4. The most common reason for not being able to quantify cases was that the D1 barrel could not be identified from the propidium iodide-stained sections. The thalamic sections were not quantified because no *LacZ*-positive cells were found in VPm nuclei.

the adult barrel cortex after whisker deprivation and to determine whether this transcription was activated by neuronal activity per se or by a more restricted set of circumstances such as those that induce plasticity.

MATERIALS AND METHODS

Animals. The CRE–*LacZ* construct contained six tandem CREs upstream of a minimal Rous sarcoma virus promoter driving β-galactosidase. CRE–*LacZ* transgenic mice from a single founder [strain 37; see Impey et al. (1996) for details] were obtained from a colony at the University of Washington and back-crossed two to six generations to wild-type C57Bl6 mice. The transgene was maintained exclusively in heterozygotes. Mice were genotyped by PCR as described (Impey et al., 1996). Experimental mice were typically postnatal day 45–50, although animals as old as 6 months were also used for this study. No obvious differences between young and old animals were noted.

Deprivation. The "single spared whisker" deprivation pattern was imposed by anesthetizing the animals briefly in metofane and then removing all the large whiskers on the right side of the muzzle, except D1, by slowly applying the minimum tension necessary to the base of each whisker. Specifically, we removed whiskers A1–A4, B1–B4, C1–C5, D2–D5, E1–E5, α, β, γ, and δ. The "unilateral deprivation" pattern was identical except that D1 was also removed. Animals were allowed to recover under a heat lamp before returning to their cages. Undeprived control animals were also anesthetized briefly the evening preceding tissue harvest; however, no whiskers were removed.

Histology and analysis. After 16 hr of deprivation or 7 d (as indicated in Results), experimental animals were anesthetized with metofane and decapitated, and the brains were rapidly dissected out into ice-cold artificial CSF containing (in mM): 124 NaCl, 4 KCl, 2 $CaCl_2$, 1 $MgSO_4$, 25 $NaCO_3$, 1.4 NaH_2PO_4, 10 D-glucose, equilibrated with 95%O_2/5%CO_2. To facilitate fixation, the two hemispheres were separated and then submerged in ice-cold fixative (3% paraformaldehyde, 0.1 M phosphate buffer) for 2 hr on ice. Tissue was then subjected to two 30 min washes at room temperature in solution A (2 mM $MgCl_2$, 10 mM PBS), one 30 min wash at room temperature in solution B (0.1 M phosphate buffer, 2 mM $MgCl_2$, 0.02% Nonidet P40, 0.01% sodium deoxycholate), and an overnight incubation at 37°C in solution C [solution B plus 5 mM potassium ferricyanide, 5 mM potassium ferrocyanide, and 0.6 mg/ml 5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside (X-gal) (Boehringer Mannheim, Indianapolis, IN)].

After X-gal histochemistry, "spared" and "deprived" hemispheres from each individual were flattened to view the entire barrel-field map in a single section. Tissue was equilibrated in sucrose before sectioning on a freezing microtome to 50 μM thickness. As has been described previously (Woolsey and Van der Loos, 1970), the barrel-field map can be visualized in layer IV by using Nissl or nuclear stains. Therefore, sections were mounted in order and stained with 0.1% propidium iodide to visualize the barrel pattern in layer IV. This technique usually enabled us to identify unambiguously the barrel corresponding to the spared whisker. Where it was difficult to establish the location of the D1 barrel, those cases were not quantified (Table 1). Other brains were sectioned coronally to examine *LacZ* expression in the thalamus.

Because commercial β-galactosidase (β-gal) antibodies generated high levels of nonspecific staining in the barrel cortex and also stained a subpopulation of cells in wild-type animals, we made use of X-gal histochemistry. This method provides the advantage of allowing us to assess global levels of transgene expression and therefore to measure changes in a particular area against a standard level of expression in the whole brain. Because of low levels of transgene expression, possible heterogeneity in transgene copy number, or variations in enzyme integrity, we were able to detect X-gal staining in only ~35% of transgenic mice. As shown in Table 1, approximately one-third of the brains showed β-gal activity, and the other two-thirds showed negligible levels of staining. Samples exhibiting low levels of β-gal activity were usually processed in parallel with brains that showed a robust X-gal reaction, so we could not attribute this negative result to differences in experimental conditions during tissue processing. Whether we blocked the brains or sectioned them and further exposed them to reactants made no difference to the levels of X-gal reactivity. To verify that this variation did not result from mistakes in genotyping, tail samples were routinely taken from deprived animals at the time of death to insure that these animals indeed carried the CRE–*LacZ* transgene. Differences in tissue processing or mistakes in animal genotyping thus cannot explain this interanimal variability. This heterogeneity of expression is in agreement with previous studies performed using animals derived from the same founder (Impey et al., 1996; Pham et al., 1999). To facilitate our analysis, we chose to concentrate our analysis of transgene activation on the group of animals showing robust β-gal activity. This subset of transgenic animals was unambiguous after X-gal staining (see Figs. 1, 2 for representative examples).

*Quantitation of CRE–*LacZ *activation.* Sections were scanned under fluorescence to identify the position of the D1 (spared) barrel. Barrel outlines were drawn with the aid of a camera lucida, and the number of X-gal-positive cells within four 105 μm^2 fields of the D1 (spared), C1 (deprived), and E1 (deprived) barrels was assessed in layer IV. Using blood vessels in the vicinity of the identified barrels, the locations of the D1, C1, and E1 barrels were identified in deep (100–150 μm below the barrel) and superficial (100–150 μm above the barrel) layers. Four 105 μm^2 fields of X-gal-positive cells were counted for each of the three barrels, in deep and superficial layers. The total number of nuclei in each field were counted and used as the denominator to determine the frequency of X-gal-positive cells within a given area. Because all sections were stained with propidium iodide and X-gal, the correspondence of CRE-mediated gene expression to barrel location could be judged extremely accurately by comparing the same sections under fluorescent and transmitted light.

Electrophysiology and analysis. The responses of 286 cells to D1 whisker stimulation were measured in seven wild-type littermates from the CRE–*LacZ* colony (147 from controls and 139 from deprived mice). A total of three mice were left undeprived, and four were deprived for 16 hr to measure the effect on D1 whisker responses in the D1 barrel. Anesthesia was induced with metofane (Arovet AG) and maintained with urethane (1.5 gm/kg body weight). Anesthetic depth was monitored throughout the experiment by testing reflexes and observing the spontaneous firing rate of neurons. Supplements of urethane were administered to maintain a state in which the hindlimb withdrawal reflex was sluggish.

The skull was thinned between 2.5–3.5 mm lateral to the midline and ~1–3 mm caudal to bregma by careful drilling. From this position it was possible to reflect a small part of the skull with a hypodermic needle and introduce the electrode through the resultant hole. The dura was left intact because the carbon fiber electrodes were able to pass through it. Cortical neurons were recorded using single barrel carbon fiber microelectrodes (Armstrong-James et al., 1980). The signal was bandpassed between 700 Hz and 7 kHz, and spikes were discriminated using a voltage window discriminator. Post-stimulus time histograms and raster plots were generated on-line and stored for later analysis using Spike 2 software (CED, Cambridge, UK). The stimulus consisted of a 200 μm deflection of the vibrissa applied ~10 mm from the face (1° deflection) and delivered at 1 Hz. The stimulator was a light-weight glass capillary touching the vibrissa attached to a fast piezoelectric bimorph wafer. All stimulus parameters were identical to those used in previous studies (Glazewski and Fox, 1996).

Neurons were sampled evenly approximately every 50 μm throughout the penetration. Cells were isolated by moving the electrode to the next position and discriminated by using its spontaneous activity. The electrode position was then adjusted by ~10–20 μm to optimize discrimination. If when a stimulus was applied a larger spike appeared, it was often, but not always, used for study instead. It was not possible to make more than three to four penetrations in the D1 barrel of each animal because of the small size of the barrel.

At the end of recording from each penetration, a small focal lesion (1.0 μA, DC, 10 sec tip negative) was made at a site of known depth in layer IV. The cortex was flattened and processed for cytochrome oxidase histology as described previously (Wong-Riley, 1979; Fox, 1992), and the location of each recording penetration was identified within the barrel field. In this way we could identify the principal vibrissa for each recorded cell.

All data were analyzed using post-stimulus time histograms and latency histograms. Response magnitude to a particular vibrissa was defined as the number of spikes per stimulus occurring between 5 and 50 msec after the stimulus minus the spontaneous activity occurring during an identical time period. The modal latency was used to describe the response latency of the neuron. For a complete description see Armstrong-James and Fox (1987).

The average response to D1 stimulation was assessed for cells in the D1 barrel by averaging the number of spikes per stimulus for all neurons recorded from the D1 barrel in each animal. The average value for each animal was then averaged again within group (deprived or undeprived) to produce a group mean. These means were compared using the Kruskal–Wallace statistical test. Further analysis was performed on the same data. Data were pooled within treatment groups, and cumulative distribution functions (CDFs) were plotted for each group subdivided by layer. The CDFs were made by summing the number of cells with a resolution of 0.1 spikes per stimulus between 0 and 3.6 spikes per stimulus. The difference between deprived and undeprived CDFs was calculated to assess the degree of shift between the curves and the significance of the difference estimated using the Kolmogorov–Smirnov two-sample test (see Fig. 5).

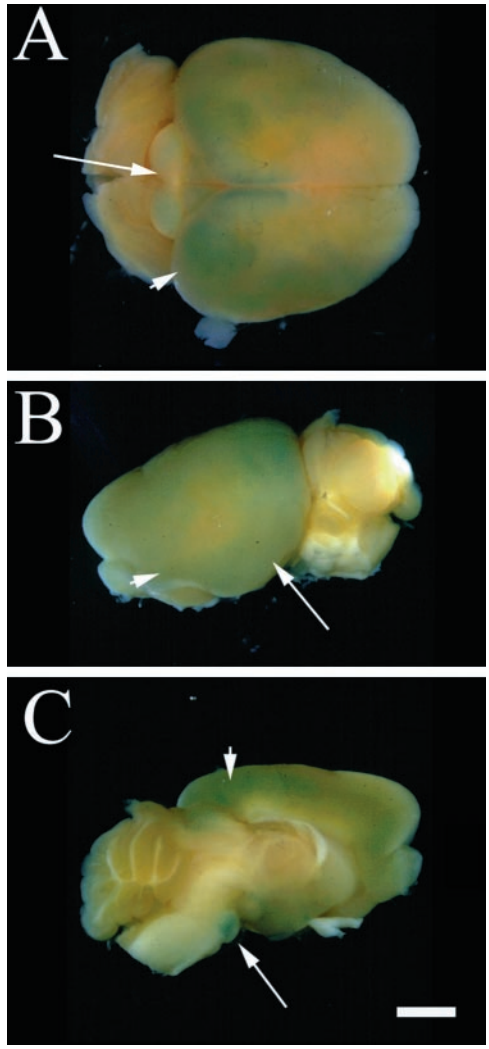

Figure 1. Basal levels of transgene expression in mouse brain. *A*, Dorsal view of cortical areas showing blue β-galactosidase reaction product reveals marked staining in visual cortex (*arrowhead*) and superior and inferior colliculi (*arrow*). *B*, Lateral view of the same brain shows staining in piriform (*arrowheads*), entorhinal cortex (*arrow*), and frontal cortex. *C*, Medial view through a bisected brain shows labeling of retrosplenial (*arrowhead*) and cingulate cortex. The pontine nuclei can also be seen to stain strongly (*arrow*). Scale bar, 2 mm.

RESULTS

Baseline levels of CRE–*LacZ* expression

To determine whether changes in CRE-mediated gene expression occur during experience-dependent plasticity, we first examined basal levels of transcription in transgenic undeprived animals. Whole brains were processed using X-gal histochemistry. This method provides the advantage of allowing us to assess global levels of transgene expression and therefore to measure changes in a particular area against a standard level of expression in the whole brain (Fig. 1).

The standard basal level of expression is shown in Figure 1, where it can be seen that several cortical areas show prominent β-gal activity. Visual structures such as the superior colliculus and visual cortex are particularly distinct (Fig. 2), with area 17 demarcated as a distinct ovoid shape at the caudal aspect of the cortex (Fig. 1*A*). Somatosensory cortex is more weakly stained than visual cortex. The inferior colliculus is strongly labeled. β-gal activity is also visible in auditory, perirhinal, and piriform cortex, as seen from the lateral aspect shown in Figure 1*B*. The medial parasagittal view shows β-gal activity in the cingulate and retrosplenial cortex as well as in the pontine nuclei (Fig. 1*C*). Although we did not perform a systematic analysis of expression throughout the CNS, from inspection of coronal sections it was clear that a subpopulation of cells consistently expressed β-gal in the hippocampus, the suprachiasmatic nucleus, and the lateral geniculate nucleus (at least four of four cases for each structure).

To quantify levels of expression, we counted the fraction of X-gal-positive cells in four undeprived animals in three areas of primary sensory cortex. We estimated that 3.4% of cells in the layer IV barrels themselves and 3.2% of cells in layers II/III directly superficial to them are β-gal-positive in these animals (Table 2). In these undeprived animals, where X-gal-positive cells were present in the barrel field, they appeared evenly distributed, with a slight tendency to outline the septal areas (data not shown).

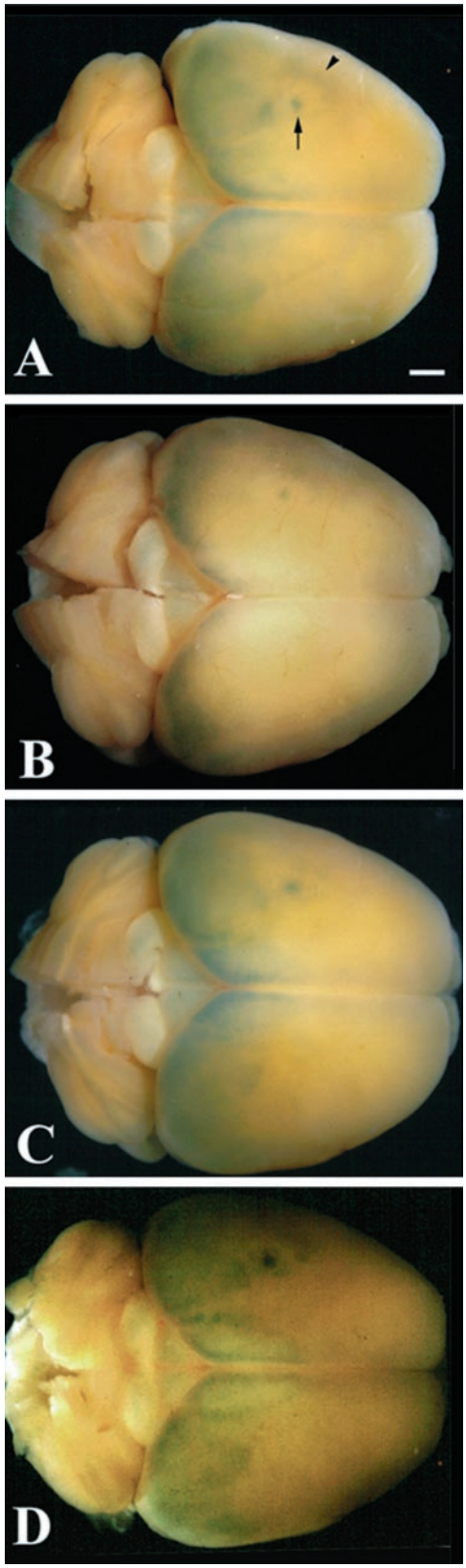

Figure 2. A period of single-whisker experience leads to upregulation of CRE-mediated gene expression in the spared barrel. Four examples are shown of CRE–*LacZ* expression in the spared D1 barrel. *A*, The D1 barrel is visible macroscopically as a *blue dot* on the cortical surface contralateral to the spared whisker (*arrow*). The *arrowhead* indicates the location of an arc of staining between the anterior and posterior barrels. There are no equivalent areas of staining in the barrel cortex of the contralateral hemisphere. Scale bar, 1 mm. *B–D*, Despite some variability in the degree of CRE–*LacZ* expression, there are several common features including the D1 barrel, the visual cortex staining, and a faintly visible arc of staining at the border of the PMBSF and the ALBSF (*A*, *arrowhead*) (also see Fig. 8). After X-gal staining, brains were sunk in 30% sucrose, which cleared the tissue and enabled visualization of expression levels below the pial surface of the brain. The right hemisphere receiving normal input from the vibrissae shows no expression over baseline levels from undeprived control animals (Fig. 1).

Table 2. The percentage of cells showing β-gal activity is shown for four undeprived animals

Layer/cortical area	SI (barrel cortex)	VI	AI
II/III	3.2 ± 1.8	15.4 ± 6.0	3.35 ± 0.07
IV	3.4 ± 1.4	16.1 ± 3.6	5.95 ± 3.61

Means and SEs are shown for cells counted in layers II/III and IV of the barrel cortex (SI) four animals, visual cortex (VI) four animals, and auditory cortex (AI) four animals. Visual cortex shows higher levels than other sensory cortices, as can also be seen macroscopically (see Figs. 1, 2).

The proportion of cells expressing β-gal in the auditory and visual cortex in coronal sections was also assessed. Auditory cortex showed levels of basal expression similar to those of barrel cortex at ~6% in layer IV and 3.4% in layers II/III (Table 2). Visual cortex had the highest frequency of X-gal-positive cells with approximately three times that observed in the corresponding layers of barrel and auditory cortex (16.1% for layer IV; 15.4% for layers II/III) (Table 2). It was particularly clear for visual cortex that most of the staining for layer IV was contained within a band running from the lower part of layer III through layer IV.

Short-term whisker deprivation and transgene activation

To address the question of whether CRE-mediated gene transcription might be induced by changes in sensory experience, we unilaterally deprived mice of all but the D1 whisker for a period of 16 hr (referred to below as "single-whisker experience"). Brains were then processed for X-gal histochemistry. The D1 barrel was macroscopically visible as a blue "dot" on the cortical surface in all 17 cases (Table 1) that showed robust β-gal activity (examples are shown in Fig. 2). That this distinct signal emanated from the D1 barrel was verified by flattening and then sectioning the neocortex in a way that preserved the barrel-field map within layer IV.

Within layer IV, we observed a clear area of β-gal activity within the D1 barrel (Fig. 3; quantified in Fig. 4). We did not find a preponderance of labeled cells at the edges versus the center of the D1 barrel. In contrast to the signal in the spared whisker's barrel, neighboring barrels showed few if any labeled cells (Fig. 3). The numbers of X-gal-positive cells in neighboring barrels appeared comparable to those in undeprived normal animals and were not statistically different (deprived barrel mean ± SEM = 2.59 ± 1.18; undeprived barrels = 3.39 ± 1.49; $t_{(6)} = 0.425$, $p > 0.5$). The spared barrel field contralateral to the deprived barrel field also showed low levels of expression (<1% X-gal-positive cells) (Fig. 3).

By identifying orienting blood vessels that passed through the barrel field in layer IV and then stacking adjacent sections, we were able to reconstruct the location of the D1 and neighboring barrel columns within both deep (layer V) and superficial layers

←

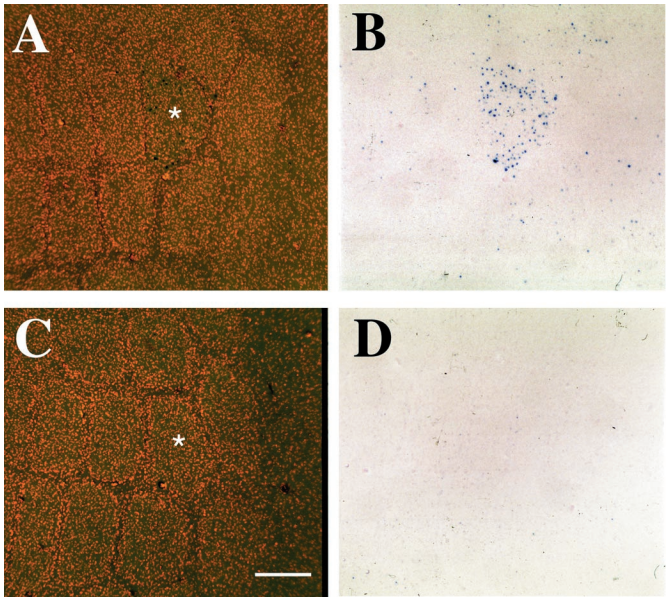

Figure 3. CRE-mediated gene expression after 16 hr of single-whisker experience. *A*, Fluorescence image of layer IV of the hemisphere corresponding to the deprived barrel field showing barrels outlined by nuclei stained with propidium iodide. The *asterisk* indicates the D1 barrel; the E1 and C1 barrels are above and below D1, respectively. *B*, Bright-field view of the same area as *A*. Tissue has been reacted with X-gal and reveals strong β-gal activity in the spared D1 barrel but not the surrounding barrels. *C*, Fluorescence image of layer IV barrel field from the undeprived hemisphere of the same animal. The D1 barrel is marked by an *asterisk*, and the orientation is the same as in *A*. *D*, Bright-field view of *C* reacted in X-gal, showing little β-gal activity. Scale bar, 150 μm.

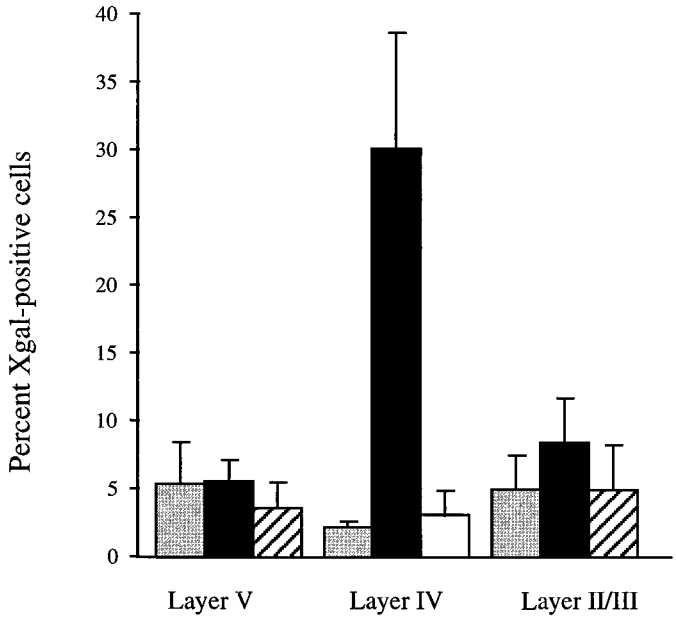

Figure 4. Frequency of X-gal-positive cells in spared versus deprived barrels, by layer. Flattened brains were sectioned tangential to the pial surface to allow identification of the individual barrels, and blood vessels were used to orient adjacent sections to this barrel map. The number of blue cells in an area within an identified barrel was counted and divided by the total number of cells within that area ($n = 4$ animals). CRE-mediated *LacZ* expression was greatest within layer IV of the spared D1 barrel (*black bars*), where labeled cells were scattered throughout the barrel. Neighboring barrels in adjacent rows were identified, and expression within these barrels was also quantified (C1, *gray bars*; E1 barrels, *hatched bars*).

(layers II/III). The frequency of X-gal-positive cells was then counted and expressed as a percentage of the total number of cells within a field (Fig. 4). It was clear from this analysis that the most substantial response to 16 hr deprivation occurred in layer IV. The D1 barrel showed greater numbers of X-gal-positive cells than either the C1 ($p < 0.01$) or E1 barrel ($p < 0.02$; one-tailed *t* test). However, the numbers of cells showing CRE-mediated gene transcription in superficial layers were similar to baseline (Fig. 4) and indistinguishable between spared and deprived barrels; D1 to C1 ($p = 0.21$) and E1 ($p = 0.24$). In deep layers, expression levels were again low in the D1 barrel column compared with layer IV (Fig. 4) and showed no consistent difference between barrel columns, with C1 showing similar numbers ($p = 0.5$) and E1 showing fewer ($p = 0.02$) X-gal-positive cells (one-tailed *t* test for all comparisons). In the control hemisphere on the opposite side, β-gal enzyme activity was not elevated in the D1 barrel and was indistinguishable from baseline levels (Figs. 3, 7).

To examine whether the thalamic nucleus that projects to the barrel field exhibited CRE-mediated gene expression, we looked through all sections containing the ventroposterior medial (VPm) nucleus of the thalamus, which was clearly visible in propidium iodide-stained sections. The location of the D1 barreloid within the VPm nucleus was known from previous electrophysiological studies in our laboratory and in others (Waite, 1973; Glazewski et al., 1998). In no case did we detect transgene activation in any of the barreloids of the VPm nucleus, including D1, despite the same set of sections showing clear expression of transgene in the spared barrel in the cortex. However, we did observe transgene expression in the lateral geniculate nucleus, in both the magnocellular and parvocellular layers of the same animals. We also encountered staining in other structures such as the hippocampus and suprachiasmatic nucleus, again in the same sections, indicating that the reactants had penetrated the tissue sufficiently to reveal expression in the VPm nucleus had it been present. Further incubation of the thalamus, sectioned to 50 μm thickness, with the reactants did not reveal any additional X-gal-positive cells (see Materials and Methods).

In summary, these results show that a restricted subpopulation of cells within the somatosensory pathway upregulates CRE–*LacZ* expression under sensory conditions that induce plasticity. It is well known that sparing just the D1 vibrissae induces plasticity (Fox, 1992; Glazewski and Fox, 1996; Wallace and Fox, 1999); however, these earlier reports were concerned with plasticity expression at 7–18 d and did not examine the very early stages of plasticity induction at 16 hr. The clear upregulation of reporter gene expression after 16 hr of deprivation therefore prompted us to determine whether corresponding changes in receptive fields occurred at this early time point.

Potentiation of spared whisker inputs after short-term deprivation

Animals were subject to 16 hr of single-whisker experience and were then anesthetized and prepared for recording. A total of 28 electrode penetrations were made in the spared whisker's barrel (D1) in seven animals. The average response of neurons in layers II/III of the spared D1 barrel after D1 whisker deflection was slightly higher in deprived versus undeprived animals (undeprived, mean D1 = 1.47 ± 0.11 spikes per stimulus; deprived, mean D1 = 1.83 ± 0.19 spikes per stimulus); however, this was not significant compared across animals ($p > 0.05$, Kruskal–

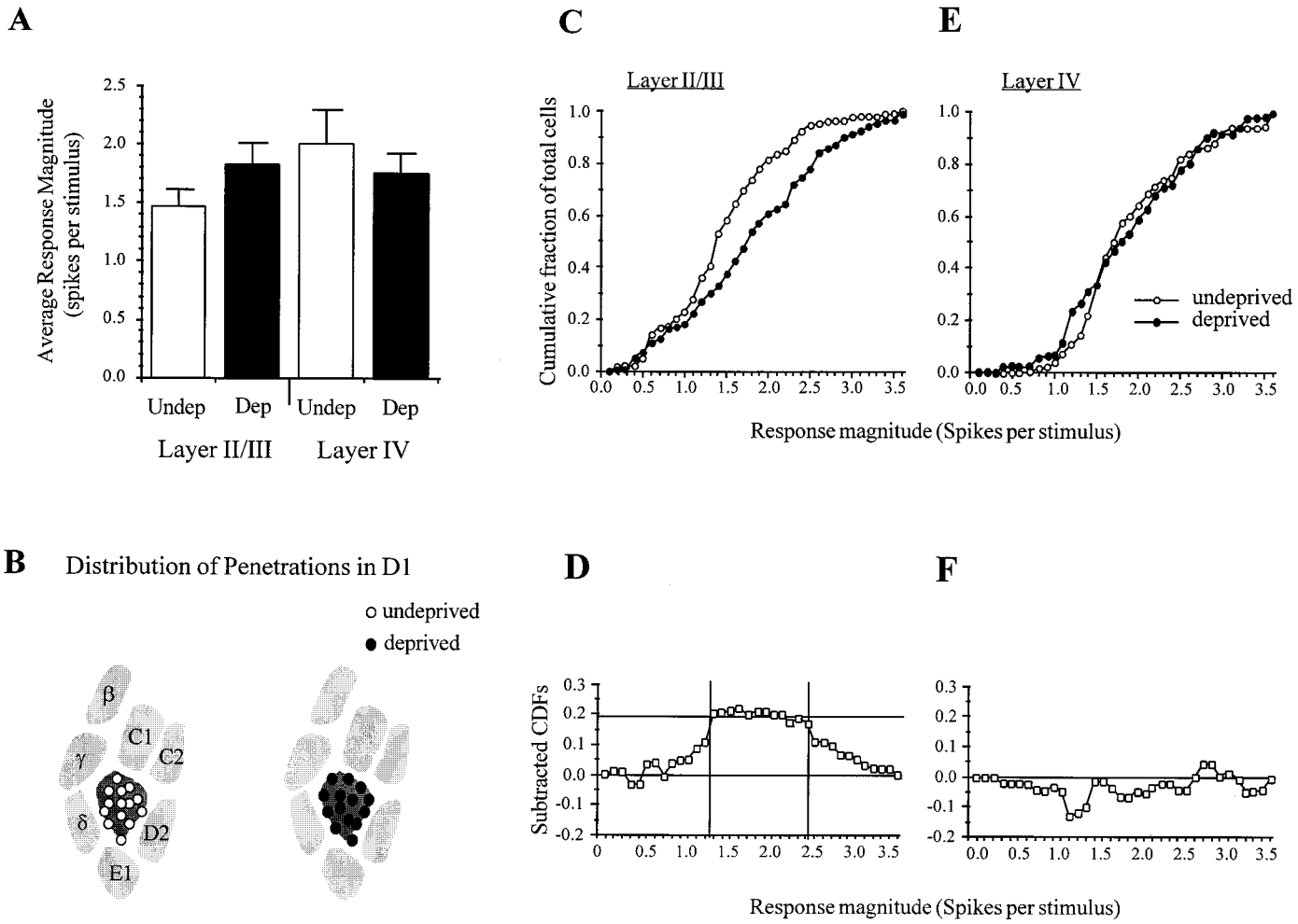

Figure 5. *In vivo* recording from 16 hr single-whisker spared versus control undeprived animals shows potentiation of spared-whisker responses. *A*, Responses (number of spikes per stimulus) of single units within layer IV and layers II/III of the D1 barrel to D1 whisker deflection from both control and single-whisker spared were recorded and averaged to generate a response magnitude average ± SEM (control undeprived, $n = 3$ animals; single-whisker spared, $n = 4$ animals). No statistically significant difference between the two experimental groups was demonstrated using this comparison. *B*, The distribution of cells within the D1 barrel is shown for deprived and undeprived cases and is indistinguishable. *C*, *E*, Responses from single units were sorted according to the magnitude of their response to D1 whisker deflection in a cumulative distribution function (*CDF*). This analysis revealed a shift in the distribution of responses of layers II/III cells ($n = 89$ cells in deprived animals and 83 cells in controls) but not layer IV cells (deprived animals, $n = 50$ cells; control animals, $n = 64$ cells). *D*, *F*, CDFs from control and single-whisker spared animals were subtracted to determine the number of cells that potentiated their responses after single-whisker experience. The difference in CDFs occurs among cells responding between 1.35 and 2.5 spikes per stimulus within layers II/III and suggests that ~20% are potentiated. No difference was observed in the subtracted CDFs for layer IV cells (*F*).

Wallace, df = 5) (Fig. 5*A*). In layer IV cells, the responses to D1 whisker stimulation appeared slightly lower in deprived animals compared with undeprived animals (deprived, mean D1 = 1.75 ± 0.14 spikes per stimulus; undeprived, mean D1 = 2.0 ± 0.26 spikes per stimulus), but again, these values were not significantly different when averaged across animals ($p > 0.05$).

One of the problems with recording from a single barrel in a mouse is that the numbers of cells sampled from each animal are few because of the small size of the barrel. It was possible to make, at most, four electrode penetrations in the D1 barrel in each animal. If potentiation to spared whisker stimulation does not occur in all the cells sampled and the sample is small, averaging cell responses for each animal may obscure the presence of a subset of potentiated cells. Therefore, to check this hypothesis, we pooled data from cells within treatment groups and calculated the CDF for each group (Fig. 5*C*,*E*). It can be seen from the CDF for layers II/III that a subpopulation of cells exhibits greater responses to stimulation of the D1 vibrissa after deprivation of all but the D1 vibrissa than would be expected in a normal animal. Subtraction of the two CDFs (Fig. 5*D*) shows that ~20% of cells are potentiated in the spared whisker's barrel if the surrounding whiskers are deprived. In contrast, the experimental and control population values overlap entirely in layer IV, and the CDF subtraction oscillates around zero, indicating that cell responsiveness does not change in this layer (Fig. 5*F*). Analysis of the CDFs revealed that the populations were different for layers II/III but not for layer IV (Kolmogorov–Smirnov test, $p < 0.01$).

Long-term whisker deprivation and transgene expression

Although CRE–*LacZ* expression was robust after 1 d of single-whisker experience, it was not clear whether this was a transient effect of sensory deprivation or whether it would be sustained

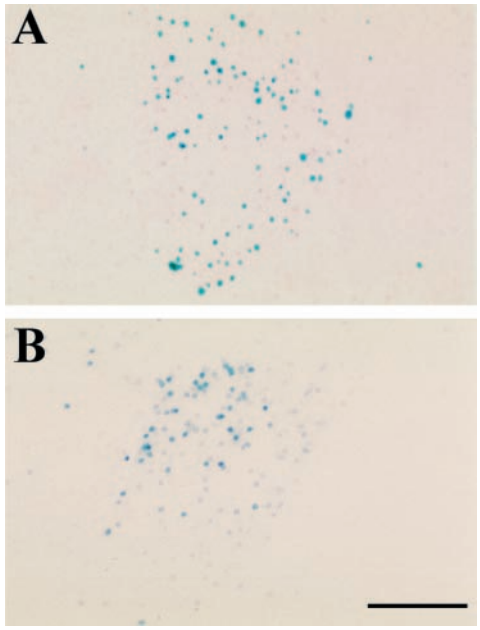

Figure 6. CRE-mediated gene expression can be sustained for long periods of single-whisker experience. An example of reporter gene expression within the spared D1 barrel at 16 hr (*A*) and 7 d (*B*) of single-whisker experience is shown. In both cases, the margin of the D1 barrel as determined by fluorescent staining of cell nuclei (data not shown) can be identified by a boundary of X-gal-positive cells. The plane of section in *B* does not include the entire D1 barrel in layer IV; adjacent sections reveal that the pattern of *LacZ* expression is roughly uniform throughout the barrel in this layer. See Results for quantification.

throughout a longer period of whisker deprivation. To clarify this issue, we subjected transgenic animals to a 7 d period of single-whisker experience and then processed their brains for X-gal histochemistry as before (Table 1).

Results were more variable after 7 d of deprivation than after 16 hr of deprivation. Although a blue dot was macroscopically visible on the brain in 17 of 17 cases with 16 hr of single-whisker experience, it was only visible in one of six cases at 7 d. Of the six cases, four were not different from undeprived animals and showed low percentages of X-gal-positive cells in layer IV (one 5%, one 4%, and two others <2%). The other two individuals from this group of six showed a higher frequency of X-gal-positive cells. In the one case (Fig. 6), the frequency of *LacZ*-expressing cells in layer IV was 33%, which is comparable to the mean level after 16 hr of single-whisker experience (30%); however, upregulation did not occur in superficial layers (<2% of cells within the D1 column expressed transgene). In deep layers we observed a less specific pattern of transgene. The percentage of X-gal-positive cells averaged 12.5% in D1, C1, and E1. In the second case, the frequency of X-gal-positive cells was above baseline in D1, but unlike the other case, was also above baseline in surrounding barrels. The percentage of X-gal-positive cells averaged 10.9% for layer IV, 9.7% for layers II/III, and 9% for layer V in the D1, C1, and E1 barrel columns.

These results demonstrate that although it is possible for CRE-mediated gene expression to persist in the D1 barrel or surrounding barrels to 7 d, it is more likely that expression returns to basal levels sometime between the end of the first day and the seventh day.

Conditions controlling transgene expression

The pattern of whisker deprivation has an effect on the type of plasticity produced in the barrel cortex. Sparing a single vibrissa in animals of this age causes potentiation of spared vibrissa responses without depression of deprived whisker responses. However, no potentiation occurs if all of the vibrissae are deprived simultaneously at this age (Glazewski et al., 1998). As a further test of the specificity of the CRE–*LacZ* upregulation for conditions that induce plasticity, we therefore deprived all of the whiskers unilaterally at the same time and looked at expression levels in the barrel cortex contralateral to the deprived vibrissae.

Mice were unilaterally deprived of all large facial vibrissae for 16 hr, and the frequency of reporter gene expression in the spared and deprived hemispheres was assessed. Under these conditions, we did not observe an upregulation of reporter gene expression in the hemisphere that retained sensory input. The percentage of X-gal-positive cells (mean ± SEM) was 3.39 ± 1.49 for control undeprived animals ($n = 4$) and 4.71 ± 2.35 on the spared side for unilaterally deprived animals ($n = 4$). These values are not significantly different ($p > 0.5$, t test).

Figure 7 summarizes the conditions that do and do not activate CRE-mediated gene expression. Levels of activation are similarly low across various conditions: in the undeprived case (7A), on the spared side after unilateral deprivation (7B), on the deprived side after unilateral deprivation (7C), and in the deprived barrels when a single whisker is spared (7D). The only condition that causes expression in these studies is the sparing of a single vibrissa in the spared vibrissa's barrel. These data support the conclusion that average levels of activity do not control CRE-mediated gene transcription in barrel cortex.

Further insight into the conditions that control gene expression were obtained from observing the small spared vibrissa barrels on the deprived side. Unilateral deprivation was performed for the large vibrissae but not for the numerous tiny whiskers around the snout. The large whiskers correspond to the posterior-medial barrel subfield (PMBSF), and the barrels representing the small whiskers toward the front of the snout correspond to the anterior-lateral barrel subfield (ALBSF). An activity contrast boundary is therefore formed at the junction of the two. At this junction we observed an arc of "spared" whisker barrels showing strong upregulation of transgene expression (three of four cases). Expression was highest in the border arc of spared barrels (C6, D6, E6), gradually decreased away from the boundary on the spared side, and decreased precipitously on the deprived side (Fig. 8*B*). This further supports the view that activity levels per se do not control CRE-mediated gene expression but activity contrasts do. The density of CRE-positive cells plotted along a roughly posterior-anterior axis confirms the visual impression that expression is highest in the first arc of spared barrels (Fig. 8*C*). A model in which the barrels are reciprocally linked by inhibitory pathways could help explain this result.

DISCUSSION

The regulation of gene transcription by synaptic events provides a mechanism by which short-lasting stimuli could be transformed into long-lasting structural changes at the synapse. The ability of the transcription factor CREB to respond to synaptic activity has therefore prompted investigators to examine the coincidence of CRE-mediated gene expression and plasticity. Here we show that CRE-mediated gene transcription is increased in the adult somatosensory cortex in response patterns of whisker deprivation that induce plasticity.

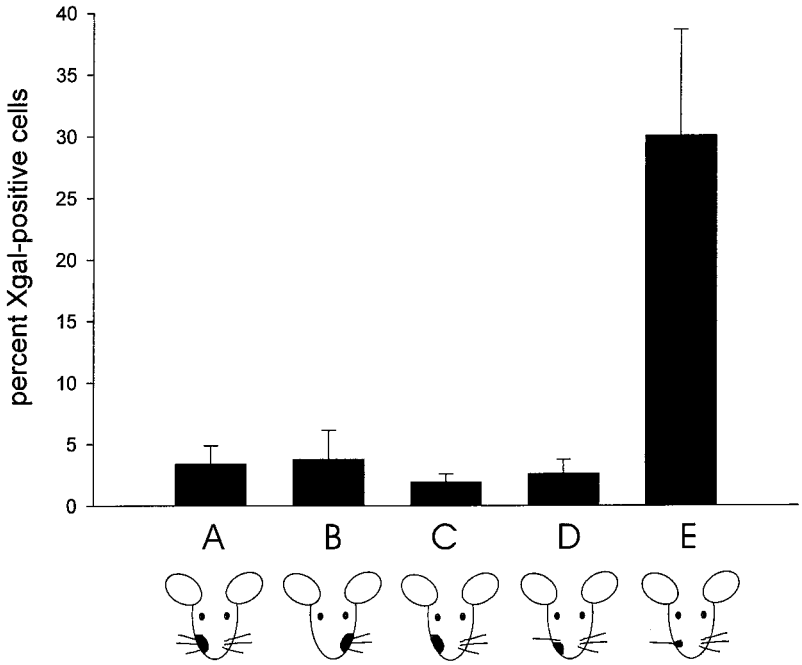

Figure 7. Upregulation of reporter gene expression is a specific response to single-whisker experience. The fraction of *LacZ*-expressing cells within layer IV was determined for (*A*) control, undeprived animals ($n = 4$), (*B*) unilateral all-whisker-deprived animals, spared side ($n = 4$), (*C*) unilateral all-whisker-deprived animals, deprived side ($n = 4$), (*D*) single-whisker spared animals, deprived barrels ($n = 4$), and (*E*) single-whisker spared animals, spared D1 barrel ($n = 4$). Data are for the 16 hr time point where deprivations are involved. A *schematic* of the deprivation conditions and whisker representation area that was quantified (*gray patch*) is shown below each bar. Values for the fraction of X-gal-positive cells did not significantly differ between control undeprived animals and sensory input-deprived areas under various deprivation conditions, suggesting that the upregulation of CRE-mediated gene expression observed with the "single-whisker spared" deprivation pattern is not a response to changes in the general level of evoked activity but is highly correlated with events known to induce potentiation of neuronal responses.

The evidence presented here is consistent with a role for transcriptional activation in the potentiation of spared sensory input. First, normal undeprived animals show negligible levels of CRE-mediated gene expression and do not exhibit any net potentiation. Second, animals with all whisker input to the PMBSF removed show no change in CRE-mediated gene expression and no potentiation (Glazewski et al., 1998). Finally, animals in which all but one whisker is removed show a highly time- and place-specific upregulation of CRE-mediated transcription and potentiation of spared whisker responses in the same cortical column. Thus, conditions that lead to potentiation lead to an upregulation in CRE–*LacZ* expression, whereas conditions that do not lead to potentiation do not lead to an upregulation of CRE–*LacZ* expression.

The conditions that induce CRE-mediated gene expression are not related to afferent levels of activity in any simple way. Expression levels are similar in spared and deprived barrels under various conditions, including unilateral deprivation and complete lack of deprivation (see Fig. 7). The levels of afferent activity produced by these two conditions are very different, yet neither potentiation nor CRE-mediated gene transcription is observed. In this system, single-whisker experience is required to induce CRE-mediated gene expression and plasticity. The conditions controlling CRE-mediated gene expression and potentiation are therefore similar to the conditions controlling ocular dominance plasticity in cat visual cortex in which binocular deprivation does not cause plasticity but monocular deprivation does (Wiesel and Hubel, 1965).

Plasticity and CRE-mediated gene expression

Previous studies in the hippocampus using this line of CRE–*LacZ* mice have found a strong correlation between the incidence of long-lasting LTP and the presence of CRE-mediated gene expression (Impey et al., 1996). This correlation was made stronger by the fact that levels of CRE-mediate gene expression were very low before induction of LTP and high during late-phase LTP. Similar arguments can be made on the basis of our studies in barrel cortex. First, we found complete correlation between the incidence of CRE-mediated gene expression and plasticity (see above). Second, levels of CRE-mediated gene expression were very low before and high after 16 hr of single-whisker experience (eightfold increase). Third, one type of correlation that is possible in barrel cortex, but not so far in hippocampus, is based on the place specificity of gene expression: only the barrel corresponding to the spared vibrissa shows upregulation of CRE-mediated gene expression after deprivation. Other locations in the barrel field immediately surrounding the barrel of the spared vibrissa remain quiescent and express low levels of the transgene both before and after deprivation.

Corroborating evidence for the role of CREB in neocortical plasticity comes from studies of α/δ CREB knockout mice. CREB is a transcription factor that is capable of linking synaptic activity to reporter gene expression, via the cAMP binding element (CRE) in the promoter of many genes. It can be phosphorylated by synaptic activity through various signal transduction pathways, including cAMP/PKA (Gonzalez et al., 1989), CAMKI/CAMKII CAMKIV (Sheng et al., 1991; Bito et al., 1997), and Erk/Rsk (Xing et al., 1996; Impey et al., 1998b). Phosphorylation of CREB allows assembly of the transcription initiation complex at the CRE site and hence induces gene transcription. Previous experiments have shown that experience-dependent plasticity and LTP in the barrel cortex are impaired in mice lacking the α/δ isoforms of CREB (Glazewski et al., 1999; Staddon and Fox, 1999). Therefore, the combination of causal evidence from the CREB knockout experiments coupled with the correlative evidence of CRE activation after whisker deprivation reported here support the conclusions that CREB activation and CRE-mediated gene transcription play a role in plasticity in the adult barrel cortex.

Mechanisms for inducing CRE-mediated gene expression

Despite the fact that the responses of layer IV neurons do not potentiate after deprivation, they must be capable of detecting the change in balance of sensory input to increase CRE–*LacZ* expression. One way in which this could occur is by a loss of

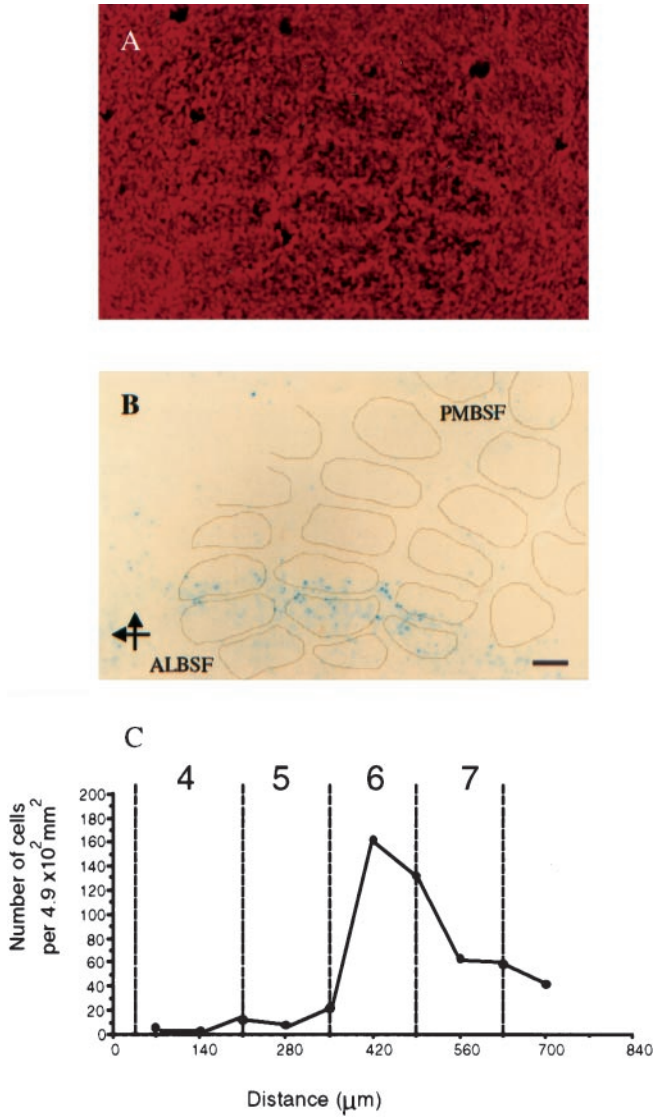

Figure 8. The spared anterior barrels show upregulation of CRE–*LacZ* after unilateral deprivation. *A*, Propidium iodide-labeled section through the barrel field showing the location of the barrels. *B*, The same section as in *A* under normal illumination showing the location of *LacZ*-positive cells that occur in the spared barrels at the border of the deprived posterior medial barrel subfield (*PMBSF*) and the anterior barrels (which were not deprived) in the anterior lateral barrel subfield (*ALBSF*). The location of the barrels has been superimposed in *outline*. Note that the number of positive cells is very low in the deprived whisker region, highest at the border, and fades back toward low levels in the spared barrels farther away from the deprived barrels. Scale bar, 100 μm. *C*, The number of cells showing CRE-mediated gene expression is quantified by counting *LacZ*-positive nuclei on either side of the spared/deprived border. The approximate locations of the four, five, six, and seven arcs of barrels are indicated by *dashed lines*. Expression is highest in the first row of spared barrels and decreases in both directions away from the spared/deprived barrel border. The contour of expression can be explained by the pattern of deprivation acting on barrels linked by reciprocal inhibitory pathways.

phasic inhibition from the surround receptive field whiskers [it could not involve a loss of tonic inhibition because layer IV neurons did not show increased responses after deprivation (Fig. 5)]. During whisking, many neighboring whiskers would normally be stimulated simultaneously, producing phasic lateral inhibition in their neighboring cortical barrels. For example, a surround vibrissa stimulated 200 msec in advance of the principal vibrissa causes substantial inhibition of the principal vibrissa response (Simons, 1985). Removing phasic inhibition by removing the surround whiskers would therefore enable a greater response to stimulation of any spared whisker. In support of this idea, recordings in awake rats have shown that the principal vibrissa response is increased when the whiskers surrounding it are removed (Kelly et al., 1999). The unusual intensity of response in the spared whisker's barrel might then be detected by voltage-gated calcium channels or NMDA receptors and thereby induce CRE-mediated gene expression.

The patterns of CRE-mediated gene expression in the D1 spared animals (Fig. 3) and at the border of the AMBSF and PMBSF in the unilaterally deprived animals (Fig. 8) are also consistent with a model whereby reciprocal lateral inhibition between the barrels controls dynamic excitability levels. The principal is similar to the familiar lateral inhibitory system operating in the retina to produce greatest activity at luminance contrast edges (Barlow and Quarles, 1975). In the present case, the greatest activity is produced at tactile borders created by the deprivation.

It is important to distinguish between the role of phasic lateral inhibition in induction versus expression of plasticity. Although phasic lateral inhibition may play a role in induction, it does not play a role in expression of plasticity. In the spared barrel, measurements of plasticity are made using single-whisker stimulation, which by definition does not produce lateral inhibition in the barrel of the principal whisker. In the surrounding barrels, changes in phasic lateral inhibition occur immediately after whisker deprivation, whereas expression of plasticity takes several days or weeks (Glazewski and Fox, 1996).

At present, it is not known whether tonic inhibition plays a role in the expression of plasticity. Recent studies have shown that excitatory pathways are more likely to be involved. It is known that αCAMKII is required for plasticity in barrel cortex, and this molecule is found in excitatory cells, not in inhibitory cells (Benson et al., 1992; Glazewski et al., 1996). Furthermore, αCAMKII is not present at the postsynaptic density of inhibitory inputs onto excitatory cells (Liu and Jones, 1996).

Does CRE-mediated gene expression play a presynaptic role in plasticity?

In this study, we observe a mismatch between the cells expressing plasticity and the cells expressing the reporter gene. Layer IV cells do not show plasticity at 16 hr but do show elevated levels of CRE-mediated gene expression, whereas the cells in layers II/III do show plasticity but do not show elevated levels of CRE-mediated gene expression. Neurons in layer IV are known to project to cells in layers II/III. Anatomical studies show that most layer IV cells have vertical projections (Woolsey et al., 1975; Harris and Woolsey 1983; Valverde, 1986; Bernado et al., 1990a,b), and synaptic terminals can be seen throughout layers II and III when individual barrel neurons are labeled (Lorente de No, 1992; Feldmeyer et al., 1999). This anatomical evidence is consistent with physiological studies showing that layers II/III cells are activated immediately after layer IV cells and before any other neurons in the column after whisker stimulation (Armstrong-James et al., 1992). This raises the intriguing possibility that CRE-mediated gene transcription plays a role in the presynaptic expression of plasticity in barrel cortex. Although it is possible that CRE-mediated gene expression does occur in layers II/III cells, but below the detection limit of our method, it is only important from the point of view of this argument that CRE-

mediated gene expression is not elevated above control levels during deprivation, and this is certainly the case.

CREB phosphorylation and CRE-mediated gene transcription have been implicated in presynaptic plasticity in other systems. In *Aplysia* (Kaang et al., 1993) as well as at the *Drosophila* neuromuscular junction (Davis et al., 1996), presynaptic CREB activation and CRE-mediated transcription can facilitate synaptic transmission. The targets of CREB activation have yet to be described in *Aplysia* and *Drosophila*, although it has been suggested that they may be related to an upregulation of release machinery or neurotransmitter synthesis/reuptake (Martin and Kandel, 1996).

The cells that express the CRE–*LacZ* transgene may similarly be activating genes whose products act to potentiate synaptic transmission. They may do so directly or indirectly via *c-fos* or *zif286*, both of which are activated by phospho-CREB and both of which can be induced in barrel cortex by sustained whisker stimulation (Melzer and Steiner, 1997). One example of an activity-regulated effector gene that has been proposed to function presynaptic to the site of plasticity is *cpg15*. This gene encodes a small membrane-bound signaling molecule, present in axons, that promotes dendritic growth in developing tectal neurons (Nedivi et al., 1998). In barrel cortex of the manipulated hemispheres, *cpg15* expression is enhanced in layer IV of the spared whisker barrel after 12 hr (Nedivi et al., 1998).

In the feline visual system, *cpg15* expression is driven by visual activity with temporal and spatial localization patterns that are consistent with a presynaptic model. Plasticity in cat visual cortex is accompanied by low levels of *cpg15* transcript in layer IV but high levels in the LGN neurons that lie presynaptic to them (Corriveau et al., 1998). In the barrel cortex, thalamic neurons are not involved in experience-dependent plasticity at this age (also see Fox, 1996; Glazewski et al., 1998; Wallace and Fox, 1999); consistent with these findings, VPm neurons did not show CRE-mediated gene expression, and the thalamic recipient neurons in layer IV did not show plasticity.

In summary, our data show that CRE-mediated gene expression is activated by patterns of whisker deprivation that are known to induce plasticity (Glazewski et al., 1998; Wallace and Fox, 1999). Potentiation of the spared whisker response is expressed in cells that lie postsynaptic to those exhibiting transgene expression rather than in the CRE-activated cells themselves. These data are consistent with observations from other organisms supporting a presynaptic role for CREB activation. The fact that only a fraction of cells in layer IV show changes in response to deprivation indicates that a subset of neurons has a lower threshold for gene induction than adjacent cells. The methods we have used here open up new ways of identifying that subset of cells and should enable identification of their unique molecular characteristics and specific connectivity.

REFERENCES

Armstrong-James M, Fox K (1987) Spatiotemporal convergence and divergence in the rat S1 "barrel" cortex. J Comp Neurol 263:265–281.

Armstrong-James M, Fox K, Millar J (1980) A method for etching the tips of carbon fibre microelectrodes. J Neurosci Methods 2:431–432.

Armstrong-James M, Fox K, Das-Gupta A (1992) The flow of excitation within rat barrel cortex on striking a single vibrissa. J Neurophysiol 68:1345–1358.

Barlow RB, Quarles DA (1975) Mach bands in the lateral eye of *Limulus*. J Gen Physiol 65:709–730.

Barth AL, Glazewski S, Hill PR, Impey S, Storm D, Fox K (1999) CRE-dependent gene expression in mouse barrel cortex following short-term whisker deprivation. J Physiol (Lond) 515P:58P.

Benson DL, Isackson PJ, Gall CM, Jones EG (1992) Contrasting patterns in the localization of glutamic acid decarboxylase and Ca2+/calmodulin protein kinase gene expression in the rat central nervous system. Neuroscience 46:825–849.

Bernado KL, McCasland JS, Woolsey TA, Strominger RN (1990a) Local intra- and interlaminar connections in mouse barrel cortex. J Comp Neurol 291:231–255.

Bernado KL, McCasland JS, Woolsey TA (1990b) Local axonal trajectories in mouse barrel cortex. Exp Brain Res 82:247–253.

Bito H, Deisseroth K, Tsien RW (1997) Ca2+-dependent regulation in neuronal gene expression. Curr Opin Neurobiol 7:419–429.

Bourtchuladze R, Frenguelli B, Blendy J, Cioffi D, Schutz G, Silva AJ (1994) Deficient long-term memory in mice with a targeted mutation of the cAMP- responsive element-binding protein. Cell 79:59–68.

Corriveau RA, Shatz CJ, Nedivi E (1998) Dynamic regulation of cpg15 expression during periods of synaptic remodeling in mammalian visual system. J Neurosci 19:7999–8008.

Dash PK, Karl KA, Colicos MA, Prywes R, Kandel ER (1991) cAMP response element-binding protein is activated by Ca2+/calmodulin- as well as cAMP-dependent protein kinase. Proc Natl Acad Sci USA 88:5061–5065.

Davis GW, Schuster CM, Goodman CS (1996) Genetic dissection of structural and functional components of synaptic plasticity. III. CREB is necessary for presynaptic functional plasticity. Neuron 17:669–679.

Davis HP, Squire LR (1984) Protein synthesis and memory: a review. Psychol Bull 96:518–559.

Diamond ME, Huang W, Ebner FF (1994) Laminar comparison of somatosensory cortical plasticity. Science 265:1885–1888.

Feldmeyer D, Egger V, Lubke J, Sakmann B (1999) Reliable synaptic connections between pairs of excitatory layer 4 neurones within a single barrel of rat somatosensory cortex. J Physiol (Lond) 521:169–190.

Fox K (1992) A critical period for experience-dependent synaptic plasticity in rat barrel cortex. J Neurosci 12:1826–1838.

Fox K (1996) The role of excitatory amino acid receptors in developmental plasticity of sensory neocortex. In: Excitatory amino acids and the cerebral cortex (Conti F, Hicks TP, eds), pp 229–244. Cambridge, MA: MIT.

Frey U, Krug M, Reymann KG, Matthies H (1988) Anisomycin, an inhibitor of protein synthesis, blocks late phases of LTP phenomena in the hippocampal CA1 region in vitro. Brain Res 452:57–65.

Glazewski S, Fox K (1996) Time course of experience-dependent synaptic potentiation and depression in barrel cortex of adolescent rats. J Neurophysiol 75:1714–1729.

Glazewski S, Chen C-H, Silva A, Fox K (1996) The requirement for aCAMKII in experience-dependent plasticity of the barrel cortex. Science 272:421–423.

Glazewski S, McKenna M, Jacquin M, Fox K (1998) The nature and origins of experience-dependent depression of vibrissae responses in rat barrel cortex. Eur J Neurosci 10:2107–2116.

Glazewski S, Barth A, Wallace H, McKenna M, Silva A, Fox K (1999) Experience-dependent plasticity in mice lacking the alpha and delta isoforms of CREB. Cereb Cortex 9:249–256.

Gonzalez GA, Yamamoto KK, Fischer WH, Karr D, Menzel P, Biggs 3rd W, Vale WW, Montminy, MR (1989) A cluster of phosphorylation sites on the cyclic AMP-regulated nuclear factor CREB predicted by its sequence. Nature 337:749–752.

Harris RM, Woolsey TA (1983) Computer-assisted analyses of barrel neuron axons and their putative synaptic contacts. J Comp Neurol 220:63–79.

Impey S, Mark M, Villacres EC, Poser S, Chavkin C, Storm DR (1996) Induction of CRE-mediated gene expression by stimuli that generate long-lasting LTP in area CA1 of the hippocampus. Neuron 16:973–982.

Impey S, Smith DM, Obrietan K, Donahue R, Wade C, Storm D (1998a) Stimulation of cAMP response element (CRE)-mediated transcription during contextual learning. Nat Neurosci 1:595–601.

Imey S, Obrietan K, Wong ST, Poser S, Yano S, Wayman G, Deloulme JC, Chan G, Storm DR (1998b) Cross-talk between ERK and PKA is required for Ca^{2+} stimulation of CREB-dependent transcription and ERK nuclear translocation. Neuron 21:495–504.

Kaang BK, Kandel ER, Grant SG (1993) Activation of cAMP-responsive genes by stimuli that produce long-term facilitation in *Aplysia* sensory neurons. Neuron 10:427–435.

Kelly MK, Carvell GE, Kodger JM, Simons DJ (1999) Sensory loss by selected whisker removal produces immediate disinhibition in the somatosensory cortex of behaving rats. J Neurosci 19:9117–9125.

Lin XB, Jones EG (1996) Localization of alpha type II calcium

calmodulin-dependent kinase at glutamatergic but not gamma aminobutyric acid (GABAergic) synapses in thalamus and cerebral cortex. Proc Natl Acad Sci USA 93:7332–7336.

Lorente de No R (1992) The cerebral cortex of the mouse (a first contribution - the "acoustic" cortex). Somatosens Motor Res 9:3–36.

Martin KC, Kandel ER (1996) Cell adhesion molecules, CREB, and the formation of new synaptic connections. Neuron 17:567–570.

Melzer P, Steiner H (1997) Stimulus dependent expression of immediate early genes in rat somatosensory cortex. J Comp Neurol 380:145–153.

Montarolo PG, Goelet P, Castellucci VF, Morgan J, Kandel ER, Schacher S (1986) A critical period for macromolecular synthesis in long-term heterosynaptic facilitation in *Aplysia*. Science 234:1249–1254.

Montminy MR, Gonzalez GA, Yamamoto KK (1990) Regulation of cAMP-inducible genes by CREB. Trends Neurosci 13:184–188.

Nedivi E, Burbach B, Svoboda K (1998a) Regulation of cpg15 expression in barrel cortex during experience-dependent receptive field plasticity. Soc Neurosci Abstr 24:634.

Nedivi E, Wu GY, Cline HT (1998b) Promotion of dendritic growth by CPG15, an activity-induced signaling molecule. Science 281:1863–1866.

Nguyen PV, Abel T, Kandel ER (1994) Requirement of a critical period of transcription for induction of a late phase of LTP. Science 265:1104–1107.

Pham TA, Impey S, Storm DR, Stryker MP (1999) CRE-mediated gene transcription in neocortical neuronal plasticity during the developmental critical period. Neuron 22:63–72.

Sheng M, Thompson MA, Greenberg ME (1991) CREB: a Ca(2+)-regulated transcription factor phosphorylated by calmodulin-dependent kinases. Science 252:1427–1430.

Simons DJ (1985) Temporal and spatial integration in the rat S1 vibrissa cortex. J Neurophysiol 54:615–635.

Simons DJ, Land PW (1987) Early experience of tactile stimulation influences organization of somatic sensory cortex. Nature 326:694–697.

Staddon JW, Fox K (1999) Long-term potentiation in barrel cortex of alpha/delta CREB deficient mice. J Physiol (Lond) 515P:57P.

Tully T (1998) Toward a molecular biology of memory: the light's coming on! Nat Neurosci 1:543–545.

Valverde F (1986) Intrinsic neocortical organization: some comparative aspects. Neuroscience 18:1–23.

Waite PME (1973) Somatotoic organization of vibrissal responses in the ventrobasal complex of the rat thalamus. J Physiol (Lond) 228:527–540.

Wallace K, Fox K (1999) Local cortical interactions determine the form of cortical plasticity. J Neurobiol 41:58–63.

Wiesel TN, Hubel DH (1965) Comparison of the effects of unilateral and bilateral eye closure on cortical unit responses in kittens. J Neurophysiol 28:1029–1040.

Wong-Riley M (1979) Changes in the visual system of monocularly sutured or enucleated cats demonstrable with cytochrome oxidase histochemistry. Brain Res 171:11–28.

Woolsey TA, Van der Loos H (1970) The structural organization of layer IV in the somatosensory region (SI) of mouse cerebral cortex. The description of a cortical field composed of discrete cytoarchitectonic units. Brain Res 17:205–242.

Woolsey TA, Dierker ML, Wann DF (1975) Mouse SmI cortex: qualitative and quantitative classification of golgi-impregnated barrel neurons. Proc Natl Acad Sci USA 72:2165–2169.

Xing J, Ginty DD, Greenberg ME (1996) Coupling of the RAS-MAPK pathway to gene activation by RSK2, a growth factor-regulated CREB kinase. Science 273:959–963.

Yin JC, Del Vecchio M, Zhou H, Tully T (1995) CREB as a memory modulator: induced expression of a dCREB2 activator isoform enhances long-term memory in *Drosophila*. Cell 81:107–115.