# Activity-Dependent Expression of *Egr1* mRNA in Somatosensory Cortex of Developing Rats

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The rat barrel field in somatosensory cortex is a well-characterized model of neocortical development, with activity-dependent and activity-independent components. *Egr1* encodes an inducible transcription factor that is required for certain forms activity-dependent plasticity. This study examines *Egr1* mRNA expression in the developing barrel field under basal conditions and after short-term deprivation or stimulation of whiskers. *Egr1* mRNA was measured with in situ hybridization at postnatal Day (P) 6, P9, P12, P15, and P21. For short-term deprivation, whiskers were trimmed close to the skin and *Egr1* mRNA was examined 3 hr later. For controlled stimulation of a single whisker, surrounding whiskers were trimmed, a wire was glued to the designated whisker, and animals were placed in an AC magnetic field pulsed at 2 Hz, 10 mT rms for 15 min. *Egr1* mRNA was examined 30 min later. At P6, basal *Egr1* mRNA in the barrel field was very low and was increased only slightly by stimulation ($P < 0.05$). At each of the later ages, there was a large increase in *Egr1* mRNA in stimulated versus deprived barrels ($P < 0.001$). *Egr1* mRNA expression after whisker stimulation increased exponentially with age through P15 ($P < 0.001$) and then declined between P15 and P21. The onset of *Egr1* responses to whisker stimulation at P9 and the striking increase in activity-dependent *Egr1* mRNA expression in the second postnatal week suggest that this transcription factor may play a role in activity-dependent processes that occur in this developmental period, such as maturation of barrel cortex circuitry. © 2004 Wiley-Liss, Inc.

Key words: ontogeny; immediate-early gene; zif268; transcription factor; barrel field

Rodent somatosensory cortex contains a map of the whisker pad, called the barrel field. Each whisker follicle is innervated by sensory fibers that project in a highly ordered manner via brainstem and thalamus to somatosensory cortex (SI). In layer IV of SI, neurons aggregate into clusters, termed barrels, that are separated from each other by zones with fewer cells, called septa (Woolsey and Van der Loos, 1970; Welker and Woolsey, 1974). Cells within each barrel are most responsive to one "principal" whisker (Welker, 1976; Simons, 1978), and the arrangement of barrels in the cortex replicates the arrangement of whiskers on the snout.

This topographic map is formed in several distinct stages, beginning with the arrival of thalamocortical axons during embryonic life (Catalano et al., 1991). The thalamocortical axon terminals and the neurons of layer IV cluster in distinct barrels during the first postnatal week (Rice et al., 1985; Erzurumlu and Jhaveri, 1990; Blue et al., 1991; Senft and Woolsey, 1991; Schlaggar and O'Leary, 1994). In the second and third postnatal weeks, oriented growth and regression of dendrites in layer IV sharpens the boundaries of the barrels (Greenough and Chang, 1988) and intrinsic cortical connections are formed (Miller et al., 2001). Although initial pattern formation has components that are independent of neuronal activity, refinement of the barrel pattern and formation of intracortical connections are activity-dependent (McCasland et al., 1992; Lendvai et al., 2000). The rat whisker-to-barrel pathway thus provides a useful developmental model of the formation and activity-dependent refinement of precise patterns of connectivity in somatosensory cortex.

The immediate-early gene *Egr1* (also called *zif268*, *Krox 24*, or *NGFI-A*) is an inducible transcription factor that is required for persistent synaptic plasticity and certain

forms of associative learning. *Egr1* expression is upregulated strongly by hippocampal long-term potentiation (Worley et al., 1993; Chinestra et al., 1994) and in behavioral models such as visual association learning (Okuno and Miyashita, 1996), during retrieval of contextual fear memory, and cued fear memory (Hall et al., 2001). Mutants lacking *Egr1* have impaired long-term memory in both spatial and nonspatial tasks (Jones et al., 2001). *Egr1* motifs have been identified in the promoter regions of several genes that may be involved in long-term potentiation, including synapsin I and II (Thiel et al., 1994; Petersohn et al., 1995) and the regulatory type IB subunit of cAMP-dependent protein kinase (Clegg et al., 1996).

Several studies suggest a role for *Egr1* in the development and activity-dependent refinement of cortical connectivity. The basal expression of *Egr1* in cerebral cortex displays significant developmental and regional variations (Herms et al., 1994). In the developing rodent visual cortex, *Egr1* expression is reduced by dark-rearing and increased upon return to ambient light (Yamada et al., 1999). In adolescent rats, environmental enrichment produces an increase in *Egr1* expression that is limited to regions of cortex that show dendritic plasticity (Wallace et al., 1995). In the adult rat, *Egr1* is induced rapidly in the barrel field by whisker stimulation (Melzer and Steiner, 1997) or tactile experience in an enriched environment (Staiger et al., 2000; Bisler et al., 2002). No studies to date, however, have examined the ontogeny of inducible transcription factors in this important developmental model. The present study examines the development of activity-dependent *Egr1* mRNA expression in the rodent barrel field.

## MATERIALS AND METHODS

### Experimental Animals

Sprague-Dawley rats (CD[SD]IGS, Charles River Laboratories) were allowed to acclimate for several days before whisker stimulation.

#### Experiment 1.

To determine whether *Egr1* and *c-fos* mRNA could be induced by whisker stimulation at postnatal Day 10 (P10, day of birth = P1), all whiskers on the right side except C1 to C3 were trimmed close to the skin and the pups (*n* = 10) were returned to their dams for 2 hr. The pups were restrained gently, and a small brush was used to deflect the C1 to C3 whiskers approximately twice per second for 15 min; mRNA expression was examined 30 min later. To obtain more controlled whisker stimulation, magnetic whisker stimulation (described below) was used in subsequent experiments.

#### Experiment 2.

Experiment 2 compared the effect of magnetic stimulation of a single whisker to tactile experience during exploration of the novel stimulator environment. In P15 rats, all whiskers on the right except D1 were trimmed and the animals were returned to their dam for 3 hr. A wire was glued to whisker D1 in half of the animals (*n* = 5), no wire was attached in the remaining littermates (*n* = 5), and all pups were placed in the magnetic stimulator in pairs for 15 min. The animals freely explored the stimulator chamber; they moved about, actively exploring and whisking during the initial period of stimulation. After initial exploration, periods of rest were commonly interspersed with additional exploration. There was some animal-to-animal variation in the amount of exploration, but differences were generally minor and no systematic differences between groups were noted. *Egr1* mRNA was examined in both hemispheres 30 min later.

#### Experiment 3.

To determine whether exploration of a novel environment increased *Egr1* mRNA expression at P15, tactile experience in the novel stimulator environment was compared to acute tactile deprivation in the same environment and to basal (home cage) conditions. In the tactile experience (tactile exp) group, all whiskers were kept intact (*n* = 4) and in the right-deprived (R-deprived) group, all whiskers on the right side were trimmed (*n* = 4). After a 3-hr recovery period, animals were placed in the magnetic stimulator chamber in pairs for 15 min. The animals actively explored the stimulator chamber, as described for Experiment two. *Egr1* mRNA expression was examined 30 min later and compared to that in littermates euthanized immediately upon removal from the home cage (naive group, *n* = 2).

#### Experiment 4.

The ontogeny of *Egr1* mRNA expression was examined at P6 (*n* = 11), P9 (*n* = 11), P12 (*n* = 6), P15 (*n* = 11), or P21 (*n* = 6). All whiskers on the right except D1 were trimmed, after a 2- to 3-hr recovery period, whisker D1 was stimulated magnetically for 15 min, and *Egr1* mRNA expression was examined in the barrel field of both hemispheres 30 min later.

### Magnetic whisker stimulation.

For magnetic whisker stimulation, pups were restrained gently while all whiskers on the right side except D1 were trimmed close to the skin and the whiskers on the left side were kept intact. A steel wire, 0.23 mm in diameter and 3 mm long, was attached with cyanoacrylate adhesive to right whisker D1, with the proximal end of the wire approximately 1 mm from the skin surface. Pups were placed in pairs in a container within a magnet coil for stimulation for 15 min. No anesthesia was required and the animals moved about the stimulus container without restraint. They were stimulated in pairs to limit stress due to isolation; they typically moved about, exploring the stimulus chamber more at the beginning of the stimulation and then resting. The wire was then removed and the pups were returned to the dams. Animals were killed by decapitation 30 min after stimulation (except for preliminary time course experiments). The cortex with underlying hippocampus was dissected away from the thalamus and striatum, flattened between foil-wrapped glass slides that were held 1 mm apart by squares of dental wax, and frozen.

The poststimulus time at which *Egr1* mRNA expression was maximal was determined in preliminary studies at P9 (*n* = 10), P12 (*n* = 10), or P15 (*n* = 10). *Egr1* mRNA was examined 20, 30, 40, 60, or 100 min after magnetic stimulation. The *Egr1* response was maximal at 20–30 min, remained significantly increased at 40–60 min, and returned to the level of the surrounding whiskers by 100 min. The time course of activity-dependent expression was comparable at the three ages

examined. In the remaining studies, *Egr1* mRNA expression was examined during the period of maximal response, 30 min after whisker stimulation.

The magnetic whisker stimulation system (Grieb's Computing Services, Syracuse, NY) was based on the design of Melzer et al. (1985). Animals were placed in a 13-cm diameter plastic container inside a wire coil that produced a 60-Hz AC magnetic field. A controller was used to turn the field on and off with adjustable frequency and field strength. Field strength, monitored with a Gauss meter, determines the force applied to the whisker and is the predominant factor affecting *Egr1* expression in the adult model (Melzer and Steiner, 1997). Field strengths from 5–15 mT were evaluated in preliminary studies. A field strength of 10 mT rms (measured at the periphery of the container) at 2 Hz for 15 min was selected for the experiments because it produced a robust, consistent *Egr1* mRNA response in the barrel field on P10.

The animal research protocol was approved by the Johns Hopkins University Animal Care and Use Committee, in accordance with PHS Policy on Humane Care and Use of Laboratory Animals.

### In Situ Hybridization

#### Oligonucleotide probes.

An antisense *Egr1* oligonucleotide probe (complementary to nucleotides 353–397 in sequence NM_012551) was used for in situ hybridization, as described previously (Wilson et al., 1998). A sense oligonucleotide with the same length and G/C content was used as a negative control. An antisense *c-fos* oligonucleotide probe (complementary to nucleotides 1,524–1,568 in sequence X06769) was also used in the first experiment. Probes were labeled with $5'-[\alpha-{}^{33}P]$dATP (Perkin Elmer Life Sciences, Inc.); incorporation was assessed with a filter-binding assay. For each experiment, sense and antisense probes were freshly labeled in parallel reactions and hybridizations were carried out in parallel.

#### Tissue preparation and hybridization.

Cryostat sections (20 $\mu$m) were cut parallel to the surface of the flattened cortex (tangential sections) and the barrel field was delineated in every third section with cytochrome oxidase (CO) histochemistry (Divac et al., 1995). Hybridization in a solution containing 2.5 pg/$\mu$l of radiolabeled oligonucleotide was carried out overnight at 42°C in sections through layer IV of the barrel field.

#### Autoradiographic analysis.

Sections were placed on BioMax MR film (Kodak), with a calibrated ${}^{14}C$ standard slide (146, American RadioChemicals, Inc.) on each film; films were exposed for 1–5 days. Densitometric analysis of autoradiographic labeling was carried out using an MCID Elite image analysis system (Imaging Research, Inc.). Barrels C1-3, D1-3, and E1-3 were delineated in cytochrome oxidase-stained sections and barrel outlines were superimposed on the corresponding autoradiographic film image. Optical density values were read within the designated barrels in three or four tissue sections from each animal, and the mean density was determined for each barrel. For most experiments, statistical analysis and data presentation focused on the stimulated D1 barrel and its immediate neighbors, C1, D2, and E1.

A standard curve was constructed from the optical density produced by ${}^{14}C$ standards on each film; this was used to adjust film exposure times so that all measured density values were within the linear response range of the film and analysis system. This provides calibrated units (in nCi/g) for comparison across films (Baskin and Stahl, 1993; Eakin et al., 1994). Except as noted, comparisons were made within single experiments.

Postnatal development of the *Egr1* mRNA response to whisker stimulation was evaluated in three hybridization experiments: at P6 (*n* = 6), P9 (*n* = 6) and P12 (*n* = 6); at P6 (*n* = 5), P9 (*n* = 5) and P15 (*n* = 5); and at P15 (*n* = 6) and P21 (*n* = 6). *Egr1* mRNA expression levels were comparable in groups of the same age across the three experiments (i.e., P6 and P9 in Experiments 1 and 2, P15 in Experiments 2 and 3). No normalization was thus required to combine the data from these three experiments, and the pooled data are shown. The autoradiograms shown in Figure 3 are derived from the first experiment for P6, P9, and P12 and from the third experiment for P15 and P21. The images were collected in a single session with constant image acquisition settings and were assembled into one figure without any alteration in contrast or brightness. Statistical analyses were carried out with SPSS. Analysis of variance and Bonferroni post-hoc tests were used to determine the significance of changes in Egr1 mRNA expression at different ages, with $P < 0.05$ considered significant. The SPSS curve estimation procedure was used to obtain an estimate of goodness of fit (Pearson correlation coefficient) for the developmental data from P6–P15, using linear and exponential curve estimation regression models.

## RESULTS

### *Egr1* and *c-fos* mRNA Expression After Whisker Stimulation at P10

In Experiment 1, whiskers C1 to C3 on the right side of P10 rats were stimulated with a brush for 15 min; *Egr1* and *c-fos* mRNA expression were examined 30 min later in coronal and tangential sections of the left barrel field cortex, which receives input from the right whisker pad. *Egr1* and *c-fos* mRNA were induced robustly by this stimulation. *Egr1* mRNA expression was high in the stimulated barrels and in the adjacent septa, but was low in the adjacent deprived rows B and D (Fig. 1b). In coronal sections, the expression of *Egr1* was higher in layer IV of the stimulated barrels than in the adjacent deprived barrels (Fig. 1c). Expression in the surrounding somatosensory cortex was moderate, with higher expression in layer IV and the superficial layers, compared to layers V–VI. The expression of *c-fos* had a different distribution. In surrounding cortex, *c-fos* expression was very low with no laminar pattern (Fig. 1d). In the stimulated row, *c-fos* mRNA was restricted to a smaller region within the barrels (i.e., there was no increase apparent in the septa surrounding the stimulated row). Under basal conditions at P10, *Egr1* mRNA expression in somatosensory cortex was moderate and could be increased by whisker stimulation, but *c-fos* mRNA expression was low and required whisker stimulation for detectable expression. For this reason, the remaining studies focused on *Egr1* mRNA expression as a more sensitive indicator of whisker-driven
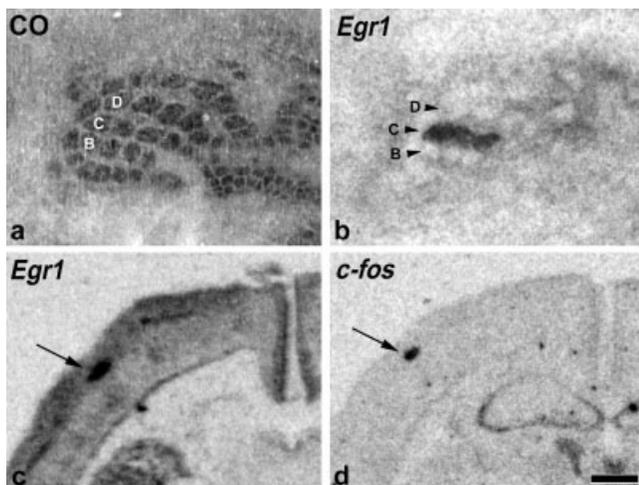
Fig. 1. Expression of *Egr1* and *c-fos* after manual stimulation of whiskers C1 to C3 at P10. **a:** Cytochrome oxidase (CO) histochemistry delineates the barrels in a tangential section. **b:** *Egr1* mRNA expression was increased in the stimulated barrels of row C, compared to the adjacent deprived rows B and D (tangential section). **c, d:** *Egr1* and *c-fos* mRNA expression are increased in layer IV (arrows) in coronal sections through the barrel field. Scale bar = 1 mm.



Fig. 2. *Egr1* mRNA expression after magnetic stimulation or tactile stimulation of left barrel D1 at P15. All whiskers except D1 in the right whisker pad were trimmed. **a:** In the left barrel field, both magnetic stimulation (Magnetic Stim) and tactile exploration (Tactile Exp) increase *Egr1* expression in barrel D1, compared to that in surrounding deprived barrels. The induction of *Egr1* mRNA in stimulated vs. deprived barrels is significantly higher after magnetic stimulation than after tactile exploration ($P < 0.001$). **b:** Expression in the right barrel field is comparable in the two groups. Comparing the two sides in the tactile exploration group, using all whiskers in the intact whisker pad produced significantly higher *Egr1* mRNA expression in right barrel D1 than that produced in left barrel D1 by whisker D1 surrounded by trimmed whiskers ($P < 0.001$).

gene expression, with the potential for increased or decreased expression.

Measurement of film optical density in the stimulated and surrounding barrels confirmed these observations, but also revealed animal-to-animal variability in the expression of *Egr1* mRNA after manual stimulation of the whiskers. For this reason, controlled stimulation with a magnetic whisker stimulator was used for the remaining studies.

## Effect of Magnetically Induced Whisker Stimulation Versus Tactile Exploration

In Experiment 2, the effect of force placed on whisker D1 by an attached wire in the magnetic stimulator was compared to tactile exploration with whisker D1 in the same environment at P15. Surrounding whiskers were trimmed in all pups, a wire was attached to right whisker D1 in five animals, and no wire was attached in five littermates. All animals were placed in the magnetic stimulation chamber, in pairs, for 15 min. Both methods of whisker stimulation produced higher *Egr1* mRNA expression in the left D1 barrel than in adjacent deprived barrels (Fig. 2a). The ratio of *Egr1* mRNA expression, however, in the stimulated D1 whisker barrel over the mean expression in adjacent deprived whisker barrels (C1, D2, E1) was significantly higher in the group with a wire glued to D1 ($164 \pm 3.8\%$ with wire vs. $137 \pm 3.9\%$ without wire, $P < 0.001$, *t*-test). To determine whether neighboring whiskers influence *Egr1* mRNA expression in the developing brain, *Egr1* mRNA expression was examined in both hemispheres of the animals with no wire on D1 (tactile exploration only). Tactile exploration with whisker D1 surrounded by intact whiskers produced signifi-

cantly higher *Egr1* expression in barrel D1 of the right barrel field (Fig. 2b), compared to that produced in left barrel D1 (Fig. 2a) by tactile exploration with whisker D1 surrounded by trimmed whiskers ($P < 0.001$, *t*-test).

## Effect of Tactile Exploration in a Novel Environment

In adult rats, tactile experience in a novel environment induces *Egr1* expression in the barrel field (Staiger et al., 2000). In Experiment 3, *Egr1* mRNA expression in the barrel field was examined in P15 rats after tactile exploration in the novel stimulator chamber environment, tactile deprivation in the same environment, or basal home cage conditions. Animals with all whiskers intact (tactile exp group) or all right whiskers trimmed 3 hr earlier (R-deprived group) freely explored the magnetic stimulator container in pairs for 15 min. *Egr1* mRNA expression, examined 30 min later in the left barrel field of these animals and in littermates euthanized immediately upon removal from the home cage (naive group), varied across whisker stimulation groups (Fig. 3; $P < 0.001$). Moderate expression of *Egr1* mRNA was observed in naive animals, higher expression in tactile exp animals ($P < 0.05$ vs. naive), and lower expression in R-deprived animals ($P < 0.05$ vs. naive, $P < 0.001$ vs. tactile exp). Tactile experience in animals that explored the novel environment of the stimulation chamber thus significantly increased *Egr1* mRNA expression. In the tactile exp group, differences among the nine barrels examined (barrels C1–3, D1–3, and E1–3, not shown) were significant ($P < 0.01$), and the relative *Egr1* expression across these barrels was generally consistent with previous reports of greater activity in caudoventral whiskers, which are longer (McCasland et al., 1991).
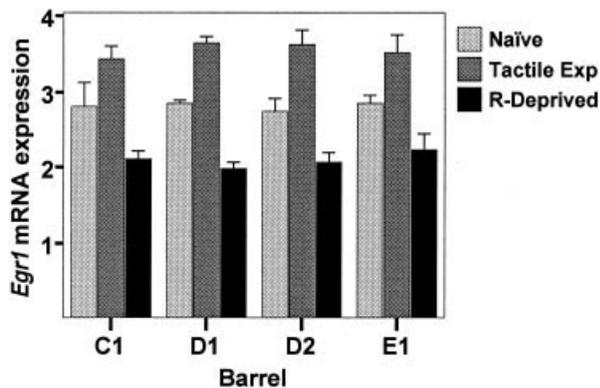
Fig. 3. *Egr1* mRNA expression produced at P15 by tactile exploration in the novel stimulator chamber environment (Tactile Exp), tactile deprivation in the same environment (R-Deprived), or basal home cage conditions (Naive). Tactile exploration produced significantly greater *Egr1* mRNA expression than that found in the naive group ($P < 0.05$) or the deprived group ($P < 0.001$). Tactile deprivation produced lower *Egr1* mRNA expression than that found in the naive group ($P < 0.05$).

## Postnatal Development of *Egr1* mRNA Expression

In Experiment 4, the postnatal development of *Egr1* mRNA expression in response to whisker stimulation was evaluated at P6 ($n = 11$), P9 ($n = 11$), P12 ($n = 6$), P15 ($n = 11$), and P21 ($n = 6$). All whiskers on the right except D1 were trimmed, whisker D1 was stimulated for 15 min, and *Egr1* mRNA was examined in the left barrel field 30 min later. Representative autoradiographic images and adjacent CO-stained sections are shown in Figure 4. The pattern of *Egr1* mRNA expression in barrel versus septa changes with age; this is most evident on the right side, which receives input from the intact left whisker pad. Septal expression is prominent at P12, expression is high in both barrels and septa by P15, and by P21 expression is higher in barrels than in septa.

Quantitative analysis of the development of *Egr1* expression is shown in Figure 5. In the magnetically stimulated left barrel field, *Egr1* mRNA expression changed significantly with age across all barrels (Fig. 5a, $P < 0.001$). In the stimulated D1 barrel or in the adjacent deprived barrels (analyzed separately), expression increased significantly between P9 and P12 and between P12 and P15 and decreased significantly between P15 and P21 ($P < 0.001$). The increase in *Egr1* mRNA expression with age from P6–P15 is fit very well by an exponential regression; data for the stimulated D1 barrel are shown with an exponential regression line in Fig. 5c ($r = 0.978$, $r^2 = 0.957$). In the surrounding deprived barrels C1, D2, and E1, exponential correlation coefficients for the increase in basal *Egr1* mRNA expression from P6–P15 ranged from 0.952–0.957.

The effect of whisker stimulation at each age was examined by comparing *Egr1* mRNA expression in the stimulated D1 barrel to that in the adjacent deprived barrels (average of C1, D2, E1). At P6, there was a small increase in *Egr1* mRNA expression between the stimu-

lated barrel and adjacent deprived barrels (15% increase, $P < 0.05$). At each of the later ages examined, stimulation caused a large increase in *Egr1* mRNA expression, compared to adjacent deprived barrels (70–95% increase, $P < 0.001$). The absolute magnitude of *Egr1* induction (the difference between D1 and the adjacent barrels) changed significantly with age ($P < 0.001$). This effect of whisker stimulation increased between P9 and P12 and between P12 and P15 ($P < 0.001$) and decreased between P15 and P21 ($P < 0.05$). Although the absolute magnitude of *Egr1* induction varied with age, the relative induction compared to adjacent deprived barrels (the ratio between D1 and the adjacent barrel average) remained approximately twofold from P9–P21.

*Egr1* mRNA expression was also evaluated in the right barrel cortex (Fig. 5b), which received tactile stimulation from the intact left whisker pad as the animals explored the stimulation chamber. *Egr1* mRNA expression increased significantly with age ($P < 0.001$); significant increases were observed between P9 and P12 and between P12 and P15 ($P < 0.001$); there was no significant difference between P6 and P9 or between P15 and P21. After tactile exploration, the increase in *Egr1* mRNA expression with age from P6–P15 is fit very well by an exponential curve; *r*-values ranged from 0.974–0.981 in barrels C1, D1, D2, and E1.

*Egr1* expression in barrel D1 after controlled magnetic stimulation of the left cortex (Fig. 5a) was also compared to that produced by tactile exploration with an intact whisker pad in the right cortex (Fig. 5b). At P6, *Egr1* mRNA expression in D1 was very low on both sides but slightly higher on the magnetically stimulated left side ($P < 0.01$); expression remained significantly higher on the left at P9 ($P < 0.01$). At P12 and P15, *Egr1* mRNA expression in barrel D1 was comparable on the two sides. By P21, tactile stimulation of the right barrel field during exploration of a novel environment produced higher *Egr1* mRNA expression than that produced by single-whisker magnetic stimulation in the left barrel field ($P < 0.01$).

## DISCUSSION

### Onset of Activity-Dependent *Egr1* Expression

*Egr1* mRNA is expressed in an age- and activity-dependent manner in the postnatal barrel cortex. At P6, basal *Egr1* mRNA expression in the barrel field is very low and the effects of stimulation or acute deprivation are barely detectable. *Egr1* expression increases in barrel cortex during the second postnatal week and becomes clearly responsive to changes in sensory input by P9. The onset of activity-dependent *Egr1* expression between P6 and P9 is consistent with the onset of cortical metabolic responses to whisker stimulation in this period (Melzer et al., 1994; Wu and Gonzalez, 1997).

*Egr1* expression after controlled stimulation of a single whisker increases exponentially from P6–P15. By P21, *Egr1* mRNA expression declines approximately 30% from the peak observed at P15. This developmental time course suggests that activity-dependent *Egr1* expression is unlikely to
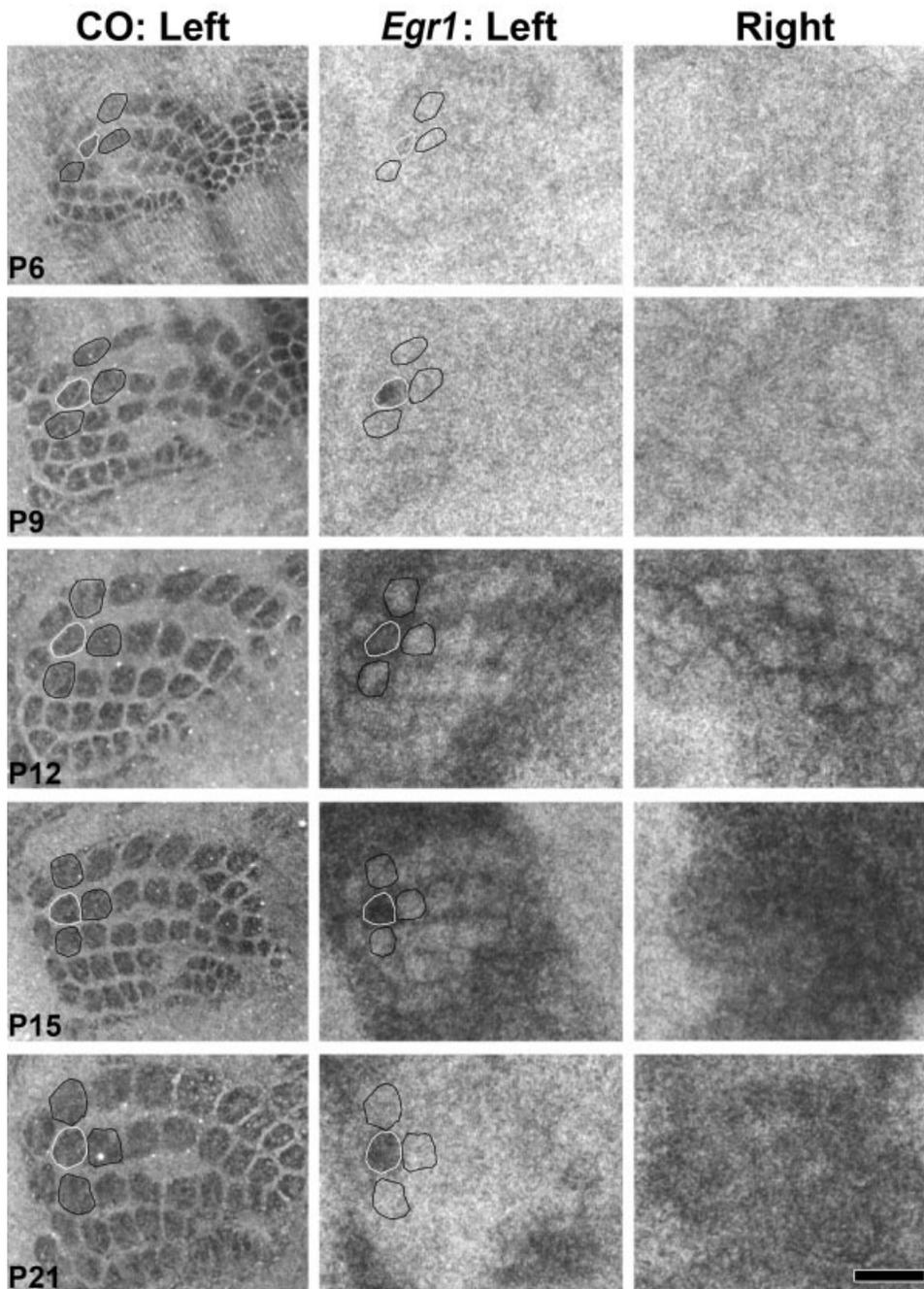
Fig. 4. Postnatal development of *Egr1* mRNA expression after whisker stimulation. All whiskers on the right except D1 were trimmed 3 hr before magnetic stimulation of whisker D1. For each age, cytochrome oxidase (CO) histochemistry was used to outline the barrels in adjacent sections (left column: white outline, barrel D1; black outline, neighboring deprived barrels). Autoradiographic images of *Egr1* mRNA in situ hybridization are shown in the left, magnetically stimulated barrel field (center column) and in the right barrel field, which receives natural tactile input from the intact left whiskers (right column; Scale bar = 1 mm). *Egr1* mRNA expression in the stimulated and surrounding deprived barrels changes with age (quantitative analysis is shown in Fig. 5); the pattern of expression in barrel vs. septa also varies with age.

play a role in early pattern formation in the barrel field but may be important in the development of somatosensory circuitry during the second and third postnatal weeks.

## Ontogeny of *Egr1* Versus Glutamate Receptors

Activity-dependent *Egr1* expression has been shown previously to be mediated by glutamate receptors. $N$-methyl-D-aspartate (NMDA) receptors have been associated specifically with regulation of *Egr1* expression (Bading et al., 1995; Lerea et al., 1995; Shan et al., 1997), AMPA receptor activation can provide the depolarization necessary to remove $Mg^{2+}$ from the NMDA receptor (NMDAR) channel, and metabotropic glutamate receptors can increase *Egr1* mRNA expression in vitro (Vaccarino et al., 1992). The temporal and spatial patterns of *Egr1* mRNA expression observed in the present study are consistent with the ontogeny of NMDA, AMPA, and metabotropic glutamate receptors reported previously (Blue and Johnston, 1995; Blue et al., 1997; Brennan et al., 1997). In particular, the spatial distribution of *Egr1* mRNA at P10 is similar to that reported for AMPA

Fig. 5. Quantitative analysis of the post-natal development of *Egr1* mRNA responses to whisker stimulation. **a:** The left barrel field receives input from the stimulated D1 and surrounding deprived whiskers. *Egr1* mRNA expression changes significantly with age ($P < 0.001$), expression increased significantly from P9–P12 and from P12–P15 and decreased significantly between P15 and P21 ($P < 0.001$). Whisker stimulation produced slightly higher *Egr1* mRNA expression in the stimulated D1 barrel than that in the surrounding deprived barrels at P6 ($P < 0.05$) and markedly higher *Egr1* mRNA expression in the stimulated barrel at later ages ($P < 0.001$). **b:** The right barrel field received natural tactile stimulation from the intact left whiskers. *Egr1* mRNA expression increased significantly with age ($P < 0.001$) and post-hoc tests showed significant increases from P9–P12 and from P12–P15 ($P < 0.001$) **c:** In the magnetically stimulated left D1 barrel, the increase in *Egr1* mRNA expression with increasing age from P6 through P15 is fit very well by an exponential regression.
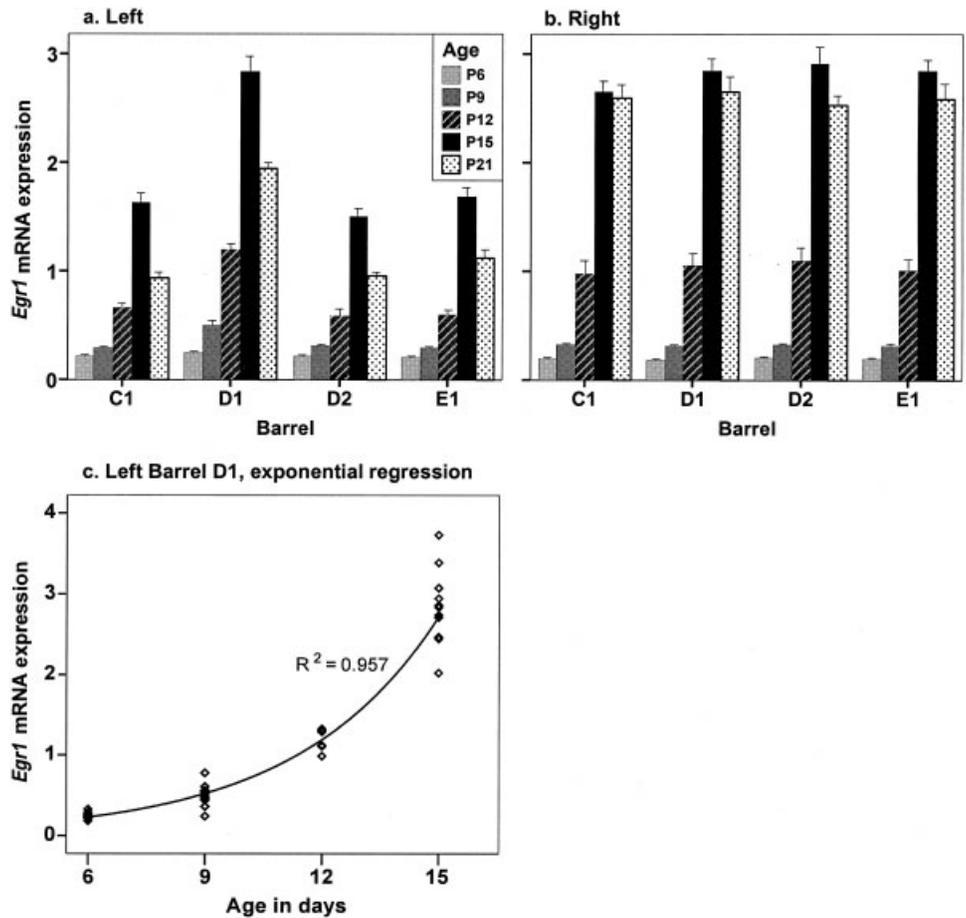
receptors at that age, with a higher density in the adjacent septa than in the stimulated barrel (Brennan et al., 1997). Between P12 and P21, the *Egr1* expression pattern reverses, with higher expression in barrel than septa; this coincides with the onset of whisking behavior, which would be expected to increase activity in the barrel. The high level of *Egr1* expression within the barrels may contribute to the activity-dependent maturation of layer IV dendritic arbors, discussed below. The restricted expression of *Egr1* mRNA within the stimulated barrel at P21 is comparable to that reported previously in adult rats (Melzer and Steiner, 1997).

## Comparison With Growth Factor Ontogeny

*Egr1* expression is also regulated by the neurotrophins nerve growth factor (NGF) and brain-derived neurotrophic factor (BDNF) (Changelian et al., 1989; Rosenblum et al., 2002), which are expressed at low to moderate levels in layer IV of somatosensory cortex at P7. Expression of NGF and BDNF mRNA and protein increases across all cortical layers during the second postnatal week (Das et al., 2001). This increase in NGF and BDNF may underlie the increase in basal *Egr1* expression observed in acutely deprived barrels during the second postnatal week.

## Relationship to Development of Barrel Field Circuitry

Basal expression of *Egr1* is very low at P6, and activity-dependent increases in expression were not clearly apparent until P9. Egr1 is thus unlikely to be involved in initial barrel pattern formation or early morphologic plasticity. During the period of maximum activity-dependent *Egr1* expression observed in this study, in the second and third postnatal weeks, dendrites of layer IV barrel neurons undergo oriented growth toward barrel centers (Greenough and Chang, 1988) and intrinsic cortical connections develop (Miller et al., 2001). Dendritic bias toward barrel centers requires activity mediated by NMDA receptors; bias is impaired in mice with targeted deletion of the NR1 NMDA receptor subunit in cortical excitatory neurons (Datwani et al., 2002). NMDA receptor activation induces *Egr1*, and induction is maximal during this phase of barrel field development. Taken together, these data suggest that *Egr1* and genes that are regulated by *Egr1* may be involved in shaping the dendritic arbors of layer IV barrel neurons. The development of intrinsic cortical circuitry is also activity dependent: infraorbital nerve transection on P7 severely limits the development of intracor-

tical connections among adjacent barrel columns (McCasland et al., 1992), and sensory deprivation during a critical period from P11–13 reduces the formation of dendritic spines and filopodia on layer II/III pyramidal neurons and impairs receptive field tuning in barrel cortex (Lendvai et al., 2000). The prominent expression of *Egr1* in layer IV during the second postnatal week suggests a possible role in development of the layer IV to layer II/III projection. Egr1 expression in layer IV neurons may increase expression of presynaptic proteins that are regulated by *Egr1*, such as the synaptic vesicle-associated proteins synapsin I and II (Thiel et al., 1994; Petersohn et al., 1995; Ferreira and Rapoport, 2002). Synapsins are required for both axonal and dendritic differentiation and development in vitro (Ferreira and Rapoport, 2002). Further studies will be required to determine whether activity-dependent *Egr1* expression is required for normal development or refinement of cortical circuitry.

## Magnetic Stimulus Versus Natural Exploratory Behavior

It is interesting to compare the development of *Egr1* responses to magnetic stimulation of a single whisker to that induced in the opposite hemisphere, which receives input from the intact whisker pad during exploration of the novel stimulator environment. At younger ages, increases in *Egr1* mRNA expression in the magnetically stimulated left barrel D1 are greater than or equal to those produced in right barrel D1 by tactile exploration. By P21, however, tactile exploration using all whiskers in an intact whisker pad produces higher *Egr1* mRNA expression than does magnetic stimulation of the D1 whisker alone, combined with trimming of the remaining whiskers on that side.

The greater induction of *Egr1* by tactile exploration using the intact whisker pad at P21 could be due to differences in stimulus intensity. Active whisking develops toward the end of the second postnatal week, and this would be expected to increase the stimulation of barrel cortex during exploratory behavior, compared to younger ages. In one of the groups for Experiment 2, however, *Egr1* mRNA was examined after tactile exploration using a single untrimmed whisker on one side or the intact whisker pad on the other side. Tactile exploration using the intact whisker pad produced a significantly greater *Egr1* response in barrel D1 than did tactile exploration using whisker D1 surrounded by trimmed whiskers.

Thalamic integration of multi-whisker input may contribute to the greater induction of *Egr1* by tactile stimulation of the intact whisker pad. Dendritic arbors in the thalamus are restricted initially to a single barreloid, but during the second postnatal week they extend into neighboring barreloids, increasing receptive field size (Brown et al., 1995; Zantua et al., 1996). Convergence of multi-whisker input has also been observed in adult rats at an earlier synaptic relay in this pathway, in the nucleus principalis of the trigeminal complex (Minnery and Simons, 2003). In adult rats, corticothalamic feedback sharpens thalamic receptive field tuning, and adjacent whisker

stimulation does not increase principal whisker responses but has a strong inhibitory effect on 2-deoxyglucose labeling or cortical unit activity (McCasland and Woolsey, 1988; Kelly et al., 1999; Temereanca and Simons, 2004). At P15, in contrast, multi-whisker input from the intact whisker pad produces greater activity-dependent *Egr1* expression in barrel D1 than does stimulation of whisker D1 in the context of deprived neighbors; this may reflect the immaturity of cortical and corticothalamic circuitry.

## CONCLUSIONS

*Egr1* mRNA expression is low during the first postnatal week, increases exponentially over the second postnatal week, and remains moderately high at the end of the third postnatal week. Induction of *Egr1* mRNA in response to whisker stimulation was barely detectable at P6 but was robust by P9. The lack of activity-dependent *Egr1* expression and low basal expression in the first postnatal week suggest that this inducible transcription factor is not likely to play a role in initial pattern formation in barrel cortex. The striking increase in activity-dependent *Egr1* mRNA expression in the second postnatal week suggests that further studies of the developmental role of this immediate-early gene should focus on activity-dependent events that occur at this time, such as refinement of barrel dendritic arbors and development of the layer IV to layer II/III projection.

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