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Stimulus-Dependent Expression of Immediate-Early Genes in Rat Somatosensory Cortex

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ABSTRACT

Using in situ hybridization histochemistry, we investigated the effects of whisker stimulation in freely moving rats on the expression of the immediate-early genes *zif 268* and c-*fos* in the barrel cortex. Whiskers equipped with metal filaments were stimulated for 5–15 minutes with a pulsating magnetic field. Such whisker stimulation resulted in increased *zif 268* and c-*fos* expression that was largely restricted to radial columns across the barrels representing the stimulated whiskers. In these columns, gene expression was elevated, to a variable degree, across the entire cortical thickness, with a distinct maximum in layer IV. The magnitude of gene expression in a barrel was proportional to the intensity of stimulation. Cellular analysis confirmed that whisker stimulation induced c-*fos* expression mostly in stellate cells of layer IV and in some pyramidal cells in other layers. However, even after the strongest stimulation, only subsets of neurons were labeled in all layers, suggesting that subpopulations of neurons with a differential genomic response to sensory input exist. These results indicate that the expression of these immediate-early genes is regulated by normal neuronal activity under physiological conditions, and suggest that such gene regulation is an integral part of neuronal function. J. Comp. Neurol. 380:145–153, 1997.

Indexing terms: c-*fos; zif 268*; sensory stimulation; whisker; barrel

Structural and functional properties of the somatosensory system have been successfully investigated with the help of metabolic imaging techniques such as the autoradiographic [^{14}C]deoxyglucose method for the measurement of local cerebral glucose metabolism (Sokoloff et al., 1977). While such techniques provide powerful tools for mapping of functional circuitry, their temporal and spatial resolution is limited. It has been especially difficult to achieve a spatial resolution that allows identification of the cells involved in the metabolic changes (Smith, 1983; Sharp et al., 1993). Recently, molecular imaging techniques with higher spatial resolution have become available. In situ hybridization histochemistry with radiolabeled cDNA or cRNA probes for the detection of mRNAs does provide cellular resolution, because mRNAs are largely confined to the cell body and the radioactive probe is relatively stably bound in the cells. Increasingly, immediate-early genes such as *zif 268* and c-*fos*, which are rapidly and transiently induced by a variety of experimental manipulations (Morgan and Curran, 1989; Sheng and Greenberg, 1990; Robertson, 1992), are being used as functional markers (Sagar et al., 1988; Dragunow and Faull, 1989; Sharp et al., 1993). Several studies have shown that, in sensory systems, the expression of such genes is affected by changes in sensory input. For example, visual deprivation resulted in decreased immediate-early gene expression in the visual cortex (Worley et al., 1991; Chaudhuri and Cynader, 1993). Similarly, somatosensory deprivation (Steiner and Gerfen, 1994) or stimulation (Hunt et al., 1987; Mack and Mack, 1992) caused a decrease or increase, respectively, in gene expression in somatosensory pathways. These results suggested that such genes are regulated by natural synaptic

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activity. However, the animals in most of these studies were either anesthetized or also subjected to stress or pain, which themselves affect immediate-early gene expression, and thus, possibly confounded the exact relationship between sensory input and gene expression. Moreover, the duration of the sensory manipulation and/or the time between treatment and assessment of gene expression (survival time) ranged between several hours and several days. Thus, little is known about short-term effects of sensory input on the regulation of these genes under physiological conditions.

In the present study, we investigated immediate-early gene expression in the somatosensory cortex after stimulation of selected whiskers in freely moving rats. Sensory input from the whisker follicles is transmitted by the trigeminal pathway, via synaptic relays in the ipsilateral brainstem and the contralateral thalamus, to a subdivision of the primary somatosensory cortex, named barrel cortex (Woolsey and Van der Loos, 1970). Barrels are cytoarchitectonic units in layer IV that represent the whiskers topologically. We examined with in situ hybridization histochemistry how stimulation of individual whiskers for a few minutes affects the expression of the immediate-early genes c-*fos* and *zif 268* in the barrel cortex. Whiskers equipped with metal filaments were stimulated with a pulsating magnetic field to mimic whisker movements as seen during sniffing behavior. The localization of immediate-early gene expression in the barrel cortex and its relationship to stimulus intensity were investigated and also compared with earlier results on changes in glucose metabolism after similar whisker stimulation. Our findings show that whisker stimulation increases immediate-early gene expression in the somatosensory cortex within minutes. Increased gene expression is largely restricted to the barrel columns that represent the stimulated whiskers, and the magnitude of gene induction is dependent on the stimulus intensity. Preliminary results of this study have been presented elsewhere (Melzer and Steiner, 1994).

MATERIALS AND METHODS

Stimulation of whiskers

Male Sprague-Dawley rats (160–180 g) were housed in groups under standard laboratory conditions with food and water provided *ad libitum*. Under light halothane anesthesia (2% in 70% N_2O/30% O_2; 5-10 minutes), pieces of Ni/Fe wire (length: 6 mm, diameter: 0.2 mm) were glued onto selected whiskers on the left side of the snout. The wire pieces were placed about 10 mm above the skin, and the distal ends of the whiskers were clipped. The animals were allowed to recover for 30 minutes prior to whisker stimulation.

The whiskers with the metal filaments were deflected by exposing the rat to a pulsating magnetic field that was generated by a copper coil (Melzer et al., 1985a). With the longitudinal axis of the magnetic field oriented horizontally, the whiskers were mostly deflected in the rostrocaudal direction. The repetition rate of the magnetic field pulses was set at approximately 7/second in an attempt to mimic the whisking frequency of a "sniffing" rat (Welker, 1964). For stimulation, the animal was placed in an acrylic cylinder (length: 15 cm, inner diameter: 14 cm) at the longitudinal center of the coil. The amplitude of whisker deflection was controlled by the strength of the magnetic field and, thus, by the voltage supplied to the coil (Melzer et al., 1985a).

The large caudal whiskers on the snout are arrayed in five rostrocaudal rows named A (dorsal) to E (ventral) (Woolsey and Van der Loos, 1970). In each row, the whiskers are numbered beginning caudally with 1. The first series of experiments (Set I) tested whether the stimulation-related expression of the two immediate-early genes was localized in the barrel columns corresponding to the stimulated whiskers. In these experiments, left whisker C2, or left whiskers C1, C2, and C3, were stimulated for 5–15 minutes with a magnetic field strength of 11.3 mT. In some rats, the surrounding whiskers on the same side were clipped close to the skin, while the whiskers of the opposite side remained intact. In other rats, all other whiskers remained intact. These animals were killed 5–30 minutes after the stimulation was stopped. The second set of experiments (Set II) tested whether the immediate-early gene induction in the barrel cortex was dependent on the intensity of stimulation. In these experiments, the left whisker C2 was stimulated for 10 minutes with one of eight field strengths varying from 1.8 mT to 15.7 mT. All other whiskers of the same side were clipped close to the skin, while those of the opposite side remained intact. Controls had a metal filament attached to left whisker C2 and were placed in the coil without magnetic field (0 mT). These rats were killed 10 minutes after the stimulation ended.

Tissue preparation

After the animals were killed with CO_2, the brains were quickly removed. Some brains of Set I were directly frozen in isopentane chilled on dry ice. In others, the cortex of the right hemisphere was carefully dissected and flattened before freezing. For Set II, only cortical flat-mounts were prepared. The tissue was stored at -20°C until sectioning. Twelve µm think sections were cut in a cryostat, either coronally or parallel to the pial surface (flat-mounts). The sections were thaw-mounted onto glass slides twice coated with gelatin, dried on a hot plate at 30°C and stored at -20°C. After warming to room temperature, the sections were post-fixed in 4% paraformaldehyde/0.9% saline for 10 minutes, incubated in a fresh solution of 0.25% acetic anhydride/0.1 M triethanolamine/0.9% saline (pH 8.0) for 10 minutes. Then they were dehydrated, defatted 2 x 5 minutes in chloroform, rehydrated, air-dried, and stored at -20°C until hybridization. Every sixth section of the flat-mounts was stained for cytochrome oxidase activity (Wong-Riley, 1979). The other sections were stained for Nissl substance after autoradiography.

In situ hybridization histochemistry and autoradiography

Gene expression was examined on series of coronal sections collected across the somatosensory cortex and on flat-mount sections through layer IV of the barrel cortex. Barrels were identified by cytochrome oxidase staining on adjacent sections. Oligonucleotide probes for c-*fos* and *zif 268* (Steiner and Gerfen, 1994) were labeled with [^{35}S]dATP as described earlier (Steiner and Gerfen, 1993). Ninety µl of hybridization buffer (Young et al., 1986) containing labeled probe (activity ca. 2×10^6 dpm) were added to each slide carrying either four coronal sections or one flat-mount section. The sections were coverslipped and incubated at 37°C for about 18 hours. After incubation, the slides were rinsed in four washes of 1× standard saline citrate (SSC) (1× SSC contains 150 mM sodium chloride and 15 mM sodium citrate). The slides were then washed

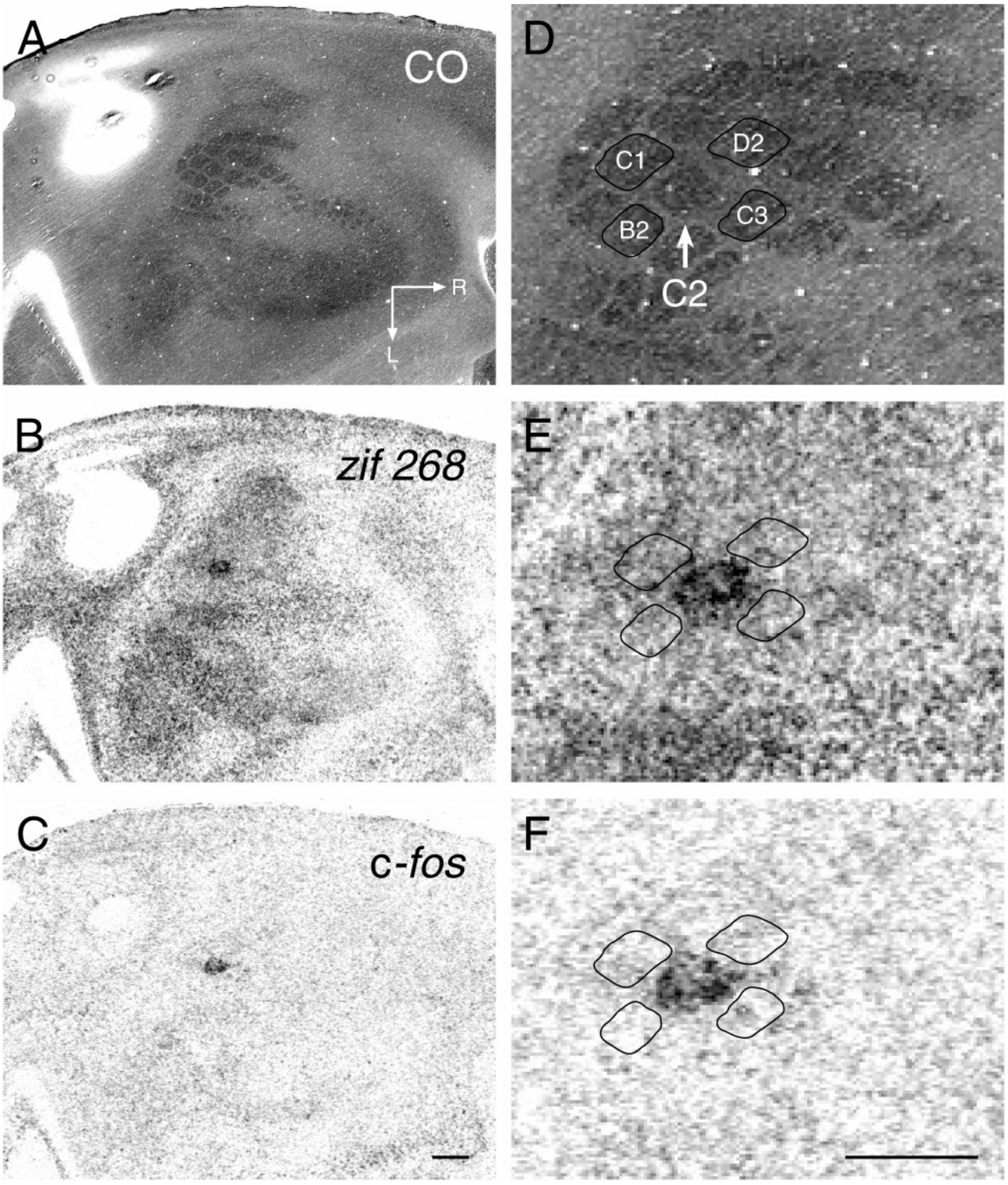

Fig. 1. Localization of immediate-early gene expression in layer IV of the barrel cortex after stimulation of a single whisker. Photomicrographs depict a section that was cut parallel to the pial surface of a cortical flat-mount (right hemisphere) and stained for cytochrome oxidase activity (CO; **A,D**), and film autoradiograms of adjacent sections probed for *zif 268* mRNA (**B,E**) or c-*fos* mRNA (**C,F**). D–F are high-power photomicrographs of the barrel field shown in A–C. This rat had left whisker C2 stimulated for 10 minutes (11.3 mT); all other surrounding whiskers were clipped (survival time after stimulation: 10 minutes). Barrels B2, C1, C3, and D2 were outlined on the cytochrome oxidase-stained section (D), and the outlines were superimposed onto the autoradiograms (E,F). Stimulation of whisker C2 produces a distinct increase in the expression of *zif 268* and c-*fos* mRNAs that is largely restricted to barrel C2. Arrows in A indicate rostral (R) and lateral (L). Scale bars = 1 mm in C, F.

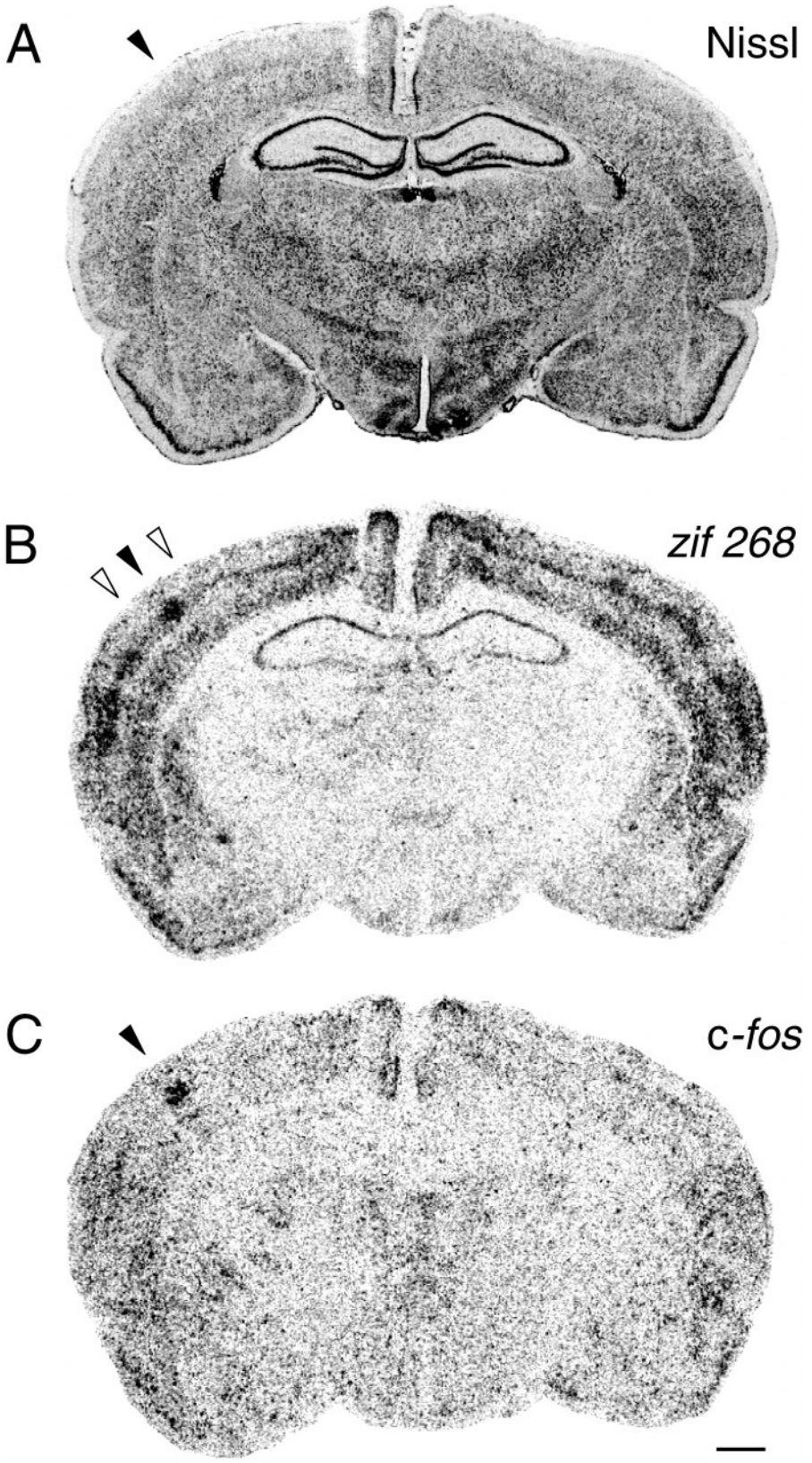

Fig. 2. Stimulation-induced immediate-early gene expression across the thickness of the barrel cortex. Photomicrographs show a coronal section stained for Nissl substance (**A**) and film autoradiograms of adjacent sections probed for *zif 268* mRNA (**B**) or c-*fos* mRNA (**C**). The sections were cut approximately perpendicular to barrel row C (Chapin and Lin, 1984). This animal had contralateral whiskers C1, C2, and C3 stimulated for 15 minutes with a magnetic field strength of 11.3 mT and was killed 5 minutes after stimulation. The surrounding whiskers were clipped 30 minutes before the onset of the stimulation, while the whiskers of the opposite side were left intact. A stimulated barrel column is indicated by filled arrowheads. The open arrowheads in B indicate the position of sensory-deprived barrel columns. Scale bar = 1 mm in C.

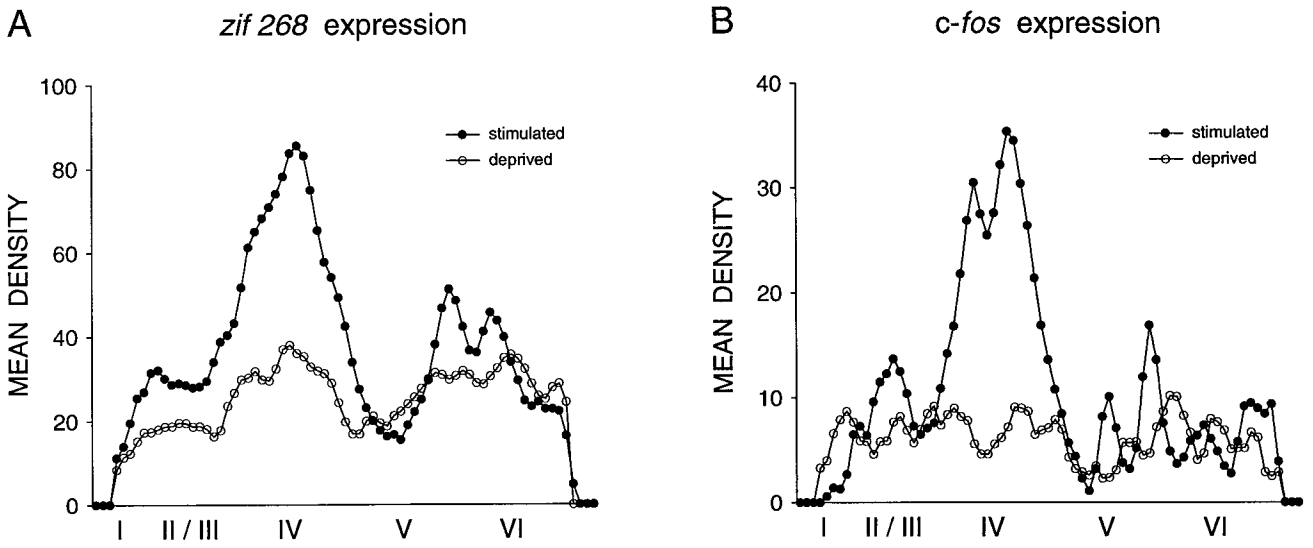

Fig. 3. Density profiles across layers I–VI of the barrel cortex after whisker stimulation. Examples of profiles for *zif 268* expression (**A**) and c-*fos* expression (**B**) were obtained from film autoradiograms of the cases displayed in Figure 2. The values shown are averaged densities (minus film background) across approximately the central 70% of the stimulated barrel column (stimulated) and of the two adjacent sensory-deprived barrel columns (deprived). Sensory stimulation-induced gene expression is maximal in layer IV, but can also be seen in superficial and deep layers. Note that the differences in location of the peaks for stimulation-induced c-*fos* and *zif 268* expression in superficial and deep layers, as shown in A and B, reflect variance in labeling between sections rather than differential expression of these genes.

three times for 20 minutes in 2× SSC/50% formamide at 40°C, followed by two 30-minute washes in 1× SSC at room temperature and a brief water rinse. Then the sections were air-dried and apposed to X-ray film (X-Omat, Kodak, Rochester, NY) for 10–30 days. After film development, the slides were dipped in nuclear emulsion (NTB3, Kodak; diluted 1:1 with water) and exposed for about 14 weeks to allow analysis at the cellular level.

Analysis of autoradiograms

The film autoradiograms were examined with a Macintosh-based image analysis system (NIH Image, Wayne Rasband, NIMH). Autoradiograms of coronal sections were used to generate optical density profiles across the six cortical layers in a barrel column. For analysis of flat-mount sections, barrels were outlined on digitized images of cytochrome oxidase-stained sections, and the outlines were superimposed onto the images of the corresponding autoradiograms. The mean optical densities (MD) of stimulated barrel C2 as well as of the four adjacent barrels (B2, C1, C3, D2) were measured, and the difference between MDs of stimulated and adjacent barrels was determined (ΔMD). Linear regressions were calculated for ΔMD values and magnetic field strengths (in mT or log mT) and tested with analyses of variance (ANOVA). In addition, ΔMD values of animals stimulated with field strengths of 5.6–15.7 mT were compared with those stimulated with 0–3.5 mT (ANOVA). The illustrations of film autoradiograms and Nissl- and cytochrome oxidase-stained sections shown in Figures 1 and 2, as well as of examples of labeled neurons displayed in Figure 4 are computer-generated images that were captured with NIH Image and contrast-enhanced to span the whole grayscale range. On film autoradiograms, the maximal hybridization signal is black.

RESULTS

Localization of gene expression

On flat-mount sections, we first determined whether stimulation-induced immediate-early gene expression in layer IV was localized in the barrel that represented the stimulated whisker. Superimposing barrel outlines from cytochrome oxidase-stained sections onto corresponding film autoradiograms demonstrated that stimulation of whisker C2 resulted in increased expression of *zif 268* and c-*fos* that was largely restricted to barrel C2 (Fig. 1). Autoradiograms of coronal sections showed that gene expression was increased in a radial column with maximal gene induction in the barrel region (Fig. 2). Density profiles across layers I–VI demonstrated that gene expression was greatest in layer IV, but was also elevated, though less, in supra- and infragranular layers, with a second peak in deep layer V/layer VI (Fig. 3). In experiments with all surrounding whiskers clipped, a greater difference in *zif 268* expression between the stimulated and the adjacent (sensory deprived) barrels was observed than in rats with the surrounding whiskers left intact (not shown). This effect was partly due to a decrease in "basal" *zif 268* expression after clipping of the surrounding whiskers (Fig. 2; see also Steiner and Gerfen, 1994). Such a difference was not detected for c-*fos* due to a lower "basal" expression of this gene.

Because of the very low basal expression of c-*fos*, this mRNA is suitable for the analysis of stimulation effects at the cellular level (Fig. 4). In agreement with the density profiles obtained from film autoradiograms, the highest proportion of labeled perikarya was detected in layer IV of the activated barrel column. According to their shape and size, the labeled neurons appeared to be mostly stellate

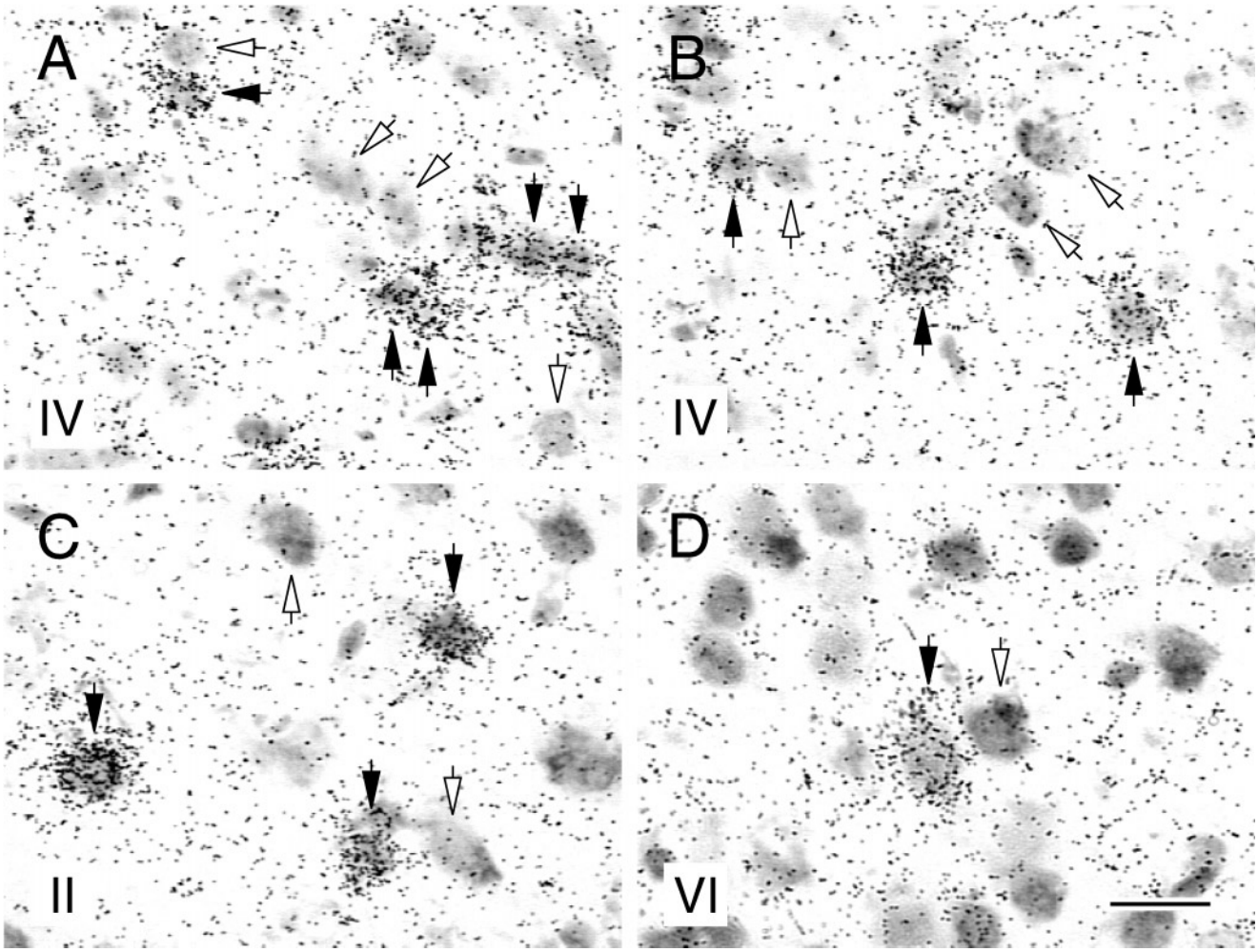

Fig. 4. Individual neurons in the barrel cortex labeled for c-*fos* mRNA after whisker stimulation. Photomicrographs depict emulsion-dipped, Nissl-stained coronal sections from a rat that had whiskers C1–3 stimulated for 15 minutes, with surrounding whiskers clipped. The panels show examples of c-*fos*-labeled neurons (clusters of silver grains; filled arrows) and of unlabeled neurons (open arrows) from layer IV of the stimulated barrel column (**A,B**), from layer II of an adjacent, sensory-deprived column (**C**) and from layer VI of the stimulated column (pyramidal cell; **D**). Labeled and unlabeled neurons are intermingled, and sometimes adjacent to each other. Scale bar in D = 20 μm and pertains to all photomicrographs.

cells. However, not every neuron in layer IV was labeled (Fig. 4A and B). Frequently, unlabeled cells were juxtaposed to heavily labeled ones. Sometimes, labeled cells appeared in clusters (Fig. 4A). This resulted in an irregular distribution of labeling that can also be appreciated on film autoradiograms (see Figs. 1 and 2). Less frequently, labeled perikarya were also seen in layers II and III and layers V and VI of the barrel column. An example of alabeled pyramidal cell in layer VI is shown in Figure 4D. In addition, in both supra- and infragranular layers, some labeled neurons were situated in adjacent, unstimulated barrel columns (Fig. 4C).

In the above experiments, rats were stimulated for 5, 10, or 15 minutes and were killed 5, 15, or 30 minutes after stimulation. No clear relationship between mRNA levels and either length of stimulation or survival time was detected (not shown). However, even the combination of the shortest time periods resulted in clearly increased gene expression.

Influence of stimulus strength on gene expression

In Set II, the magnetic field strength was varied to investigate the relationship between stimulus intensity and gene expression in the barrel. The expression of both *zif 268* and c-*fos* in layer IV increased with increasing stimulus strength (Fig. 5). The mRNA levels in the rats stimulated with field strengths of 5.6–15.7 mT were significantly greater than those in the rats stimulated with 0–3.5 mT (Fig. 5). For both genes, the stimulus-response relationship can be described with a linear regression, at either semi-logarithmic (log mT) (*zif 268*: $r = 0.66$, $P < 0.01$; c-*fos*: $r = 0.74$, $P < 0.01$; Fig. 5) or linear scaling (*zif 268*: $r = 0.64$, $P < 0.05$; c-*fos*: $r = 0.76$, $P < 0.01$; not shown).

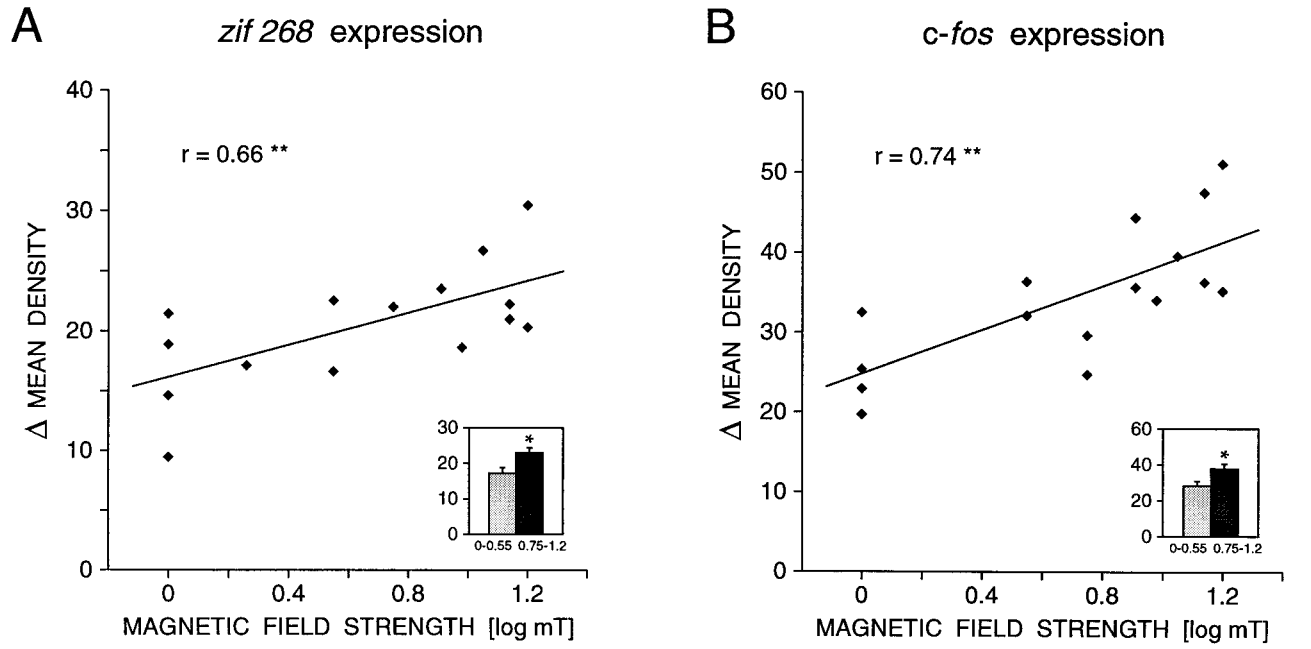

Fig. 5. Relationship between stimulus intensity and immediate-early gene expression in layer IV of the barrel cortex. Mean density values of individual animals measured on film autoradiograms of flat-mount sections are shown for *zif 268* expression (**A**) and c-*fos* expression (**B**). Rats had whisker C2 stimulated for 10 minutes with a magnetic field strength varying between 0 and 15.7 mT. The surrounding whiskers were clipped 30 minutes before stimulation. The values represent the differences between the density measured in the stimulated barrel C2 and the averaged densities of the surrounding barrels B2, C1, C3, and D2 (Δ mean density). An increase in magnetic field strength resulted in an increase in expression of *zif 268* and c-*fos* mRNAs in the stimulated barrel. This relationship can be described with a linear regression. Insets: Pooled data (mean ± SEM) from field strengths 0–3.5 mT and 5.6–15.7 mT. r, Regression coefficient. $^{**}P < 0.01$, $^{*}P < 0.05$.

DISCUSSION

In the present study, we investigated the expression of the immediate-early genes *zif 268* and c-*fos* in the barrel cortex induced by stimulation of selected whiskers in the freely moving rat. Natural stimulation of whisker follicle receptors was mimicked by exposing the rat to a pulsating magnetic field with a pulse rate similar to the whisking frequency in a sniffing rat. Our findings demonstrate that such sensory stimulation is sufficient to induce, within a few minutes, immediate-early gene expression in the barrel column that represents the stimulated whisker. This response is strongest in layer IV and is proportional to the stimulus intensity. Single cell analysis revealed that stimulation-induced gene expression occurred in subsets of neurons in the barrel column. These findings show that tactile sensory input under physiological conditions results in increased gene expression in the somatosensory cortex in an activity-dependent manner. Thus, such gene regulation seems to be an integral part of normal neuronal function and may serve homeostatic purposes.

Localization of stimulation-induced immediate-early gene expression in the barrel cortex

Barrels can be readily visualized with staining for cytochrome oxidase activity (Wong-Riley and Welt, 1980) and, thus, provide excellent morphological landmarks for the localization of autoradiographic signals produced by whisker stimulation. Using these landmarks on cortical flat-mount sections, we first demonstrated that, in layer IV, whisker stimulation-induced immediate-early gene expression is localized in the barrel representing the stimulated whisker. This correspondence confirms the well-established isomorphism between whiskers and barrels and is in accord with numerous electrophysiological studies demonstrating that neurons in a barrel respond predominantly to deflection of the "principal" whisker, i.e., the whisker represented by that barrel (e.g., Welker, 1971; Simons, 1978; Ito, 1985; Nussbaumer and Van der Loos, 1985; Armstrong-James and Fox, 1987; Armstrong-James et al., 1992; Simons et al., 1992; Welker et al., 1993). However, these studies also showed that the receptive fields of barrel neurons are generally larger as stimulation of one or more adjacent whiskers can also evoke excitatory responses in the "principal" barrel. In contrast, our results demonstrate that changes in gene expression in layer IV after stimulation of a single whisker are restricted to the principal barrel. It is not clear why excitatory responses in adjacent barrels are apparently not accompanied by increased gene expression. This effect may be related to the sparsity of direct barrel-to-barrel connections in the rat (Hoeflinger et al., 1995), or to the types of excitatory responses (e.g., Armstrong-James et al., 1993; Welker et al., 1993) and/or the cell types (see below) that show these responses in the surrounding barrels.

Coronal sections revealed an increase in gene expression throughout the principal barrel column, with maximal gene induction in layer IV and a second, lesser peak in deep layer V/layer VI. This profile is similar to that of

metabolic activation in deoxyglucose studies (Hand, 1981; Melzer et al., 1985a; Chmielowska et al., 1986), and mirrors the distribution of the termination sites of thalamocortical afferents (Bernardo and Woolsey, 1987; Jensen and Killackey, 1987). In infra- and supragranular layers, receptive fields tend to be larger than in layer IV (e.g., Simons, 1978; Armstrong-James and Fox, 1987). Consistently, in layers II/III, V, and VI, some labeled neurons were also found in columns adjacent to the stimulated one.

Relationship between stimulus intensity and gene expression

Sensory deprivation by whisker clipping results in decreased immediate-early gene expression in the barrel cortex (Steiner and Gerfen, 1994), indicating that normal sensory input to the cortex maintains a certain level of gene expression. One purpose of the present study was to investigate how stimulation of whiskers would increase immediate-early gene expression, and whether this gene regulation would be dependent on the intensity of sensory stimulation. Thus, the question of how closely such gene expression can "image" afferent input/firing rates under physiological conditions was addressed. Our findings demonstrate that the expression of *zif 268* and c-*fos* in the barrel cortex is, indeed, a function of the strength of the sensory stimulation, and that in situ hybridization histochemistry is sensitive enough to depict such changes in gene regulation under physiological conditions. Deoxyglucose studies with the same stimulus parameters as used here showed that the metabolic activation of a barrel is also dependent on the strength of whisker stimulation (Melzer et al., 1985a; Melzer et al., 1985b). However, this metabolic activity presumably reflects mostly presynaptic spiking in thalamocortical afferents (Mata et al., 1980; Kadekaro et al., 1985). In contrast, in the present study, immediate-early gene induction revealed responses to whisker stimulation in postsynaptic cortical neurons. Our finding that gene induction in the barrel is proportional to the stimulus intensity is in harmony with single-unit recordings that showed that barrel neurons respond to increasing whisker excursions with an increase in the number of spikes per stimulus (Armstrong-James and Fox, 1987). Taken together, these findings indicate that the regulation of immediate-early genes and spike rate are closely related in such neurons.

Evidence for different subpopulations of cortical neurons

One intriguing observation of the present study is that not all neurons in the stimulated barrel column responded to whisker stimulation with increased immediate-early gene expression. Even after the strongest stimulation, only subsets of the neurons were labeled. Responsive and "unresponsive" neurons were often adjacent to each other. This effect was seen in all layers, but was most distinct in layer IV. We cannot rule out that with a further increase in stimulation, by increasing either duration or magnitude, more neurons would have been recruited. Although not investigated systematically here, some observations argue against this possibility. For example, the duration of stimulation was varied between 5 and 15 minutes without discernable effect. Moreover, even after stimulation with the highest intensities, many neurons remained unlabeled. These observations suggest that subpopulations of cortical neurons with differential genomic responses to sensory input exist. Presently, the basis for such differential gene induction is unclear. Several potential explanations are conceivable. For example, neurons could differ in their connectivity. Parallel circuits with different functions have been shown in the visual cortex (Van Essen et al., 1992; Casagrande and Kaas, 1994). Alternatively, these neurons could differ in phenotypical characteristics such as excitatory amino acid receptors (Armstrong-James et al., 1993) or calcium-buffering mechanisms (Chaudhuri et al., 1995), given the importance of calcium-dependent second messenger pathways in the regulation of immediate-early genes (Morgan and Curran, 1989; Sheng and Greenberg, 1990). Two types of stellate cells that differ in their response to sensory stimulation have indeed been demonstrated in the somatosensory cortex (Mountcastle, 1957; Simons and Carvell, 1989; Agmon and Connors, 1992; Welker et al., 1993). Future experimentation will have to address the basis for the observed differential genomic response to sensory input.

Functional implications

Previous work has shown that long-term sensory manipulations influence the levels of enzymes such as cytochrome oxidase (Wong-Riley and Welt, 1980; Land and Simons, 1985) or glutamic acid decarboxylase (Welker et al., 1989; Akhtar and Land, 1991) in the affected barrels. The present study demonstrates that behaviorally-relevant sensory input can activate the immediate-early genes *zif 268* and c-*fos* in the barrel cortex within minutes. These immediate-early genes encode transcription factors that regulate the activity of other genes (Morgan and Curran, 1989; Sheng and Greenberg, 1990; Robertson, 1992) and thus are part of the cellular machinery that mediates the transsynaptic regulation of the expression of enzymes/proteins. Thus, a role of these genes in long-term neuroadaptive changes has previously been stressed. However, our results indicate that these genes are also involved in homeostatic gene regulation that is necessary to maintain normal neuron function.

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