

A set of genes expressed in response to light in the adult cerebral cortex and regulated during development

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ABSTRACT Activity-dependent plasticity is thought to underlie both formation of appropriate synaptic connections during development and reorganization of adult cortical topography. We have recently cloned many candidate plasticity-related genes (CPGs) induced by glutamate-receptor activation in the hippocampus. Screening the CPG pool for genes that may contribute to neocortical plasticity resulted in the identification of six genes that are induced in adult visual cortical areas in response to light. These genes are also naturally induced during postnatal cortical development. CPG induction by visual stimulation occurs primarily in neurons located in cortical layers II–III and VI and persists for at least 48 hr. Four of the visually responsive CPGs (*cpg2*, *cpg15*, *cpg22*, *cpg29*) are previously unreported genes, one of which (*cpg2*) predicts a “mini-dystrophin-like” structural protein. These results lend molecular genetic support to physiological and anatomical studies showing activity-dependent structural reorganization in adult cortex. In addition, these results provide candidate genes the function of which may underlie mechanisms of adult cortical reorganization.

In recent years there has been an accumulation of physiological and anatomical evidence for functional and structural changes in the adult cortex in response to peripheral manipulation (1–9). These experiments suggest that the adult cortex can undergo more dynamic changes than previously thought (10, 11). Studies of long-term potentiation in the hippocampus have shown that long-term changes in synaptic transmission involve activation of gene expression (12, 13). The hippocampus and neocortex, sharing structural similarities and common forms of synaptic plasticity (14), also probably share molecular mechanisms that underlie the capability of changing in response to activity. A complementary approach to physiological and anatomical studies of adult cortical plasticity would be the identification of genes and characterization of the gene induction that produces activity-induced, cortical reorganization.

Activation of glutamate receptors is thought to be involved in synaptic reorganization during development (15–17) and in some forms of synaptic plasticity in the adult hippocampus (12). Three hundred candidate plasticity-related genes (CPGs) were cloned in our laboratory as genes induced in the hippocampal dentate gyrus by the potent glutamate analog kainate (18). The CPG pool represents a potential source of genes that may be relevant to many activity-dependent phenomena. The CPGs were therefore screened for genes that may contribute to activity-dependent synaptic plasticity in the neocortex. To this purpose we tested CPGs for induction in the adult neocortex in response to physiological stimulation. The same CPGs were also tested for expression postnatally in the developing rat neocortex. This screen has resulted in the identification of six CPGs (four of them previously unreported), the expression of which is modulated by changes in the level of neural

activity regulated by light in the adult cortex and also regulated during postnatal cortical development. The existence of a set of genes with these properties, the time course of their induction, their layer localization, and the nature of some of the encoded proteins are all consistent with anatomical and physiological studies demonstrating activity-dependent structural reorganization in adult cortex.¶

METHODS

Animals. All rats used were male Wistar of The Weizmann Institute. Dark-adaptation was produced by placing normally reared, 8-week-old rats in the dark for 2 weeks. Rats were then sacrificed without light exposure or exposed to constant light for the following times: 1/2 hr, 1 hr, 2 hr, 4 hr, 6 hr, 12 hr, 24 hr, or 48 hr. For time-course experiments four or five animals were used for each time point; for developmental studies three to seven animals were used at each age. Animals were sacrificed by guillotine decapitation; brains were then dissected out, frozen on powdered dry ice, and kept at -70°C until sectioning. Kainate injections for identification of “cortical CPGs” were as described (18).

In Situ Hybridization. Freshly frozen brains were sectioned by cryostat ($10\ \mu\text{m}$) and mounted on Superfrost/plus microscope slides (Fisher Scientific). Sections were dried, fixed in 4% (wt/vol) paraformaldehyde, washed in phosphate-buffered saline, dehydrated in ethanol, air-dried, and then stored desiccated at -70°C . Before hybridization, slides were pretreated (at room temperature, unless otherwise stated) with 0.2 M HCl (20 min); deionized distilled water (DDW) (5 min); $2\times$ standard saline citrate (30 min at 70°C); DDW (5 min); the next prehybridization treatments, from proteinase K to air-drying slides for 1 hr, were conducted essentially as described (19). RNA probes were synthesized with an RNA transcription kit (Stratagene) and ^{35}S -labeled UTP (Amersham) in the sense and antisense orientations, using linearized pBluescript (Stratagene) templates of CPG cDNAs. Hybridizations were done as described (19). Posthybridization washes were as follows: 5 hr at 50°C in 50% (wt/vol) formamide/ $1\times$ salt solution (19)/10 mM dithiothreitol; 15 min at 37°C in TNE (10 mM Tris, pH 7.5/0.5 M NaCl/1 mM EDTA); 30 min at 37°C in TNE/RNase A at $20\ \mu\text{g}/\text{ml}$ (Sigma); 30 min at 37°C in TNE; and finally overnight at 50°C in 50% formamide/ $1\times$ salt solution. Slides were then dehydrated, air-dried, processed for autoradiography as described (19), and exposed for 3–6 days at 4°C .

In situ hybridizations of time-course experiments with *cpg2*, *cpg15*, *cpg22*, and *cpg29* probes were done in duplicate in two experiments on two brains for each 1/2-hr, 1-hr, 2-hr, and 4-hr time point; in three experiments on three brains for each 12-hr, 24-hr and 48-hr time point; and in three experiments on four brains for normal adult control, dark-adapted (no light expo-

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Abbreviation: CPGs, candidate plasticity-related genes.

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¶The sequence described in this paper has been deposited in the GenBank data base (accession no. X95466).

Table 1. CPG induction by light in visual cortex

Gene	Basal expression	Light induction	
		Time	Place
<i>cpg2</i>	Low, all layers not reduced by D	4 hr → 48 hr	Layers II, III, IV, and VI
<i>cpg15</i>	All layers reduced by D	Recovered from D in 4 hr; 12 hr → 48 hr	Layers II, III, IV, and VI
<i>cpg22</i>	None	1/2 hr → 4 hr	Layers II, III, and VI
<i>cpg29</i>	None	2 hr → 48 hr	Layers II, III, and VI
<i>zif268</i>	None	1/2 hr → 2 hr 12 hr → 48 hr	All layers All layers
<i>rheb</i>	All layers reduced by D	2 hr → 48 hr	All layers

D, placed in dark for 2 weeks.

sure) control, and 6-hr time point. *In situ* hybridizations with *zif268* and *rheb* probes were done in duplicate in two experiments on two brains for each time point. Developmental *in situ* hybridizations with *cpg29*, *zif268*, and *rheb* probes were done in duplicate in two experiments on two brains for each age; with a *cpg2* probe were performed in duplicate, in two experiments on three brains for each age; and with *cpg15* and *cpg22* were performed in duplicate, in three experiments on five brains for each age.

RESULTS

Fifty-one CPGs were screened by *in situ* hybridization for expression in adult rat neocortex. Twenty-nine CPGs were found to be expressed in the adult neocortex either at a basal level or after induction by the glutamate analog kainate (data not shown). These "cortical CPGs" were then tested for their activation by the physiological stimulus light. Six CPGs were induced by light in visual cortex (Table 1; examples in Figs. 1–3), of these, four are previously unreported genes: *cpg2*, *cpg15*, *cpg22*, and *cpg29*. The other two light-induced CPGs are known immediate early genes: *zif268* and *rheb* (20, 21). *rheb* was previously reported to be induced in the cortex by seizure (21), whereas *zif268* was shown to be down-regulated in the cortex by activity blockade (22, 23) and transiently induced by light (22).

For a time course of CPG induction by light, adult rats were placed in the dark for 2 weeks and then exposed to constant light for increased periods of time (1/2 hr, 1 hr, 2 hr, 4 hr, 6 hr, 12 hr, 24 hr, or 48 hr) until euthanasia. *cpg22* was found to have no basal expression in the adult cortex (Fig. 1a). Its light-induced expression starts 1 hr after light exposure, stays constant throughout the time points tested, and persists until 48 hr (Fig. 1a). *cpg15* basal expression in the cortex of normal untreated rats is reduced by dark treatment (Fig. 1b). Expression returns to normal levels 4 hr after light exposure, staying

constant until 8 hr later. At this time a "patch" of increased expression appears, which becomes distinct at 24 hr and persists until 48 hr (Fig. 1b). Light-induction of the immediate early gene *zif268* is biphasic (Fig. 1c). The early phase starts 1/2 hr after light exposure, remains elevated until 2 hr, and is then undetectable until 12 hr, when a late phase of induction begins, and persists until 48 hr (Fig. 1c).

The immediate early genes *zif268* and *rheb* are induced in all cortical layers. *zif268* is particularly prominent in layer IV (Fig. 1c). *cpg29* is induced primarily in cortical layers II–III and VI (Fig. 2a). *cpg22* is induced in cortical layers II–III and VI (Fig. 1a) but is more prominent in layers III and VI (Fig. 1a 24-hr time point and Fig. 2a). *cpg2* and *cpg15* are both clones that exhibit basal expression in cortical layers II–IV and VI (Fig. 3a N and Fig. 1b N, respectively), and induction by light of both is seen in all four of these layers (Figs. 2a and 3a; Figs. 1b and 2a). However, for perspective as to the level of expression of *cpg2* and *cpg15* in layer IV in relation to layers II–III and VI, compare to *zif268* (Fig. 1c). In some brains, induction of *cpg15* and *cpg22* in parts of the light-induced patch appears stronger in layer III than in layer II (Fig. 2a, Fig. 1a 24H, Fig. 1b 24H and 48H). Although each clone has an individual pattern of light-induced expression, layers II–III and VI feature most prominently in this response. Induction by light in these layers is common to all light-induced CPGs.

To gain insight into the possible function of these visually responsive CPGs, we obtained the full-length sequences of three of the four previously unreported genes: *cpg2*, *cpg15*, and *cpg22*. The CPG2 sequence predicts a 941-amino acid protein, consisting of nine repeated motifs of ≈100 amino acids (Fig. 3c). These motifs resemble the 25 repeated motifs of the same size found in the Duchenne muscular dystrophy protein dystrophin and in spectrin [(24); Fig. 3b]. Each motif predicts a triple-helical segment that is the basic structural unit of the dystrophin rod-like structure (24). CPG2 also con-

Table 2. Adult and developmental expression of light-induced CPGs

Gene	Predicted protein	Adult expression		Postnatal developmental expression	
		Basal	Kainate	Time	Place
<i>cpg2</i>	Structural	Ctx	Ctx	1d→10d	Ctx
<i>cpg15</i>	Secreted	Ctx + T	DG + CA1 + Ctx	1d = 2w after 2w	PiCtx + Hip + T Nctx
<i>cpg22</i>	?	None	Ctx	1d→2w	Ctx
<i>cpg29</i>	?	None	Ctx	1d→7d	Nctx
<i>zif268</i>	Transcription factor	Ctx	Ctx	2w	Ctx
<i>rheb</i>	Small G protein	Entire brain	Entire brain	1d = 2w	Entire brain

Hip, Hippocampus; Ctx, entire cortex, including neocortex, hippocampus, piriform, and entorhinal cortices; D, placed in dark for 2 weeks; T, thalamus; PiCtx, piriform cortex; DG, dentate gyrus; Nctx, neocortex; CA1, hippocampal region CA1. Arrows indicate maintained induction between the times specified.

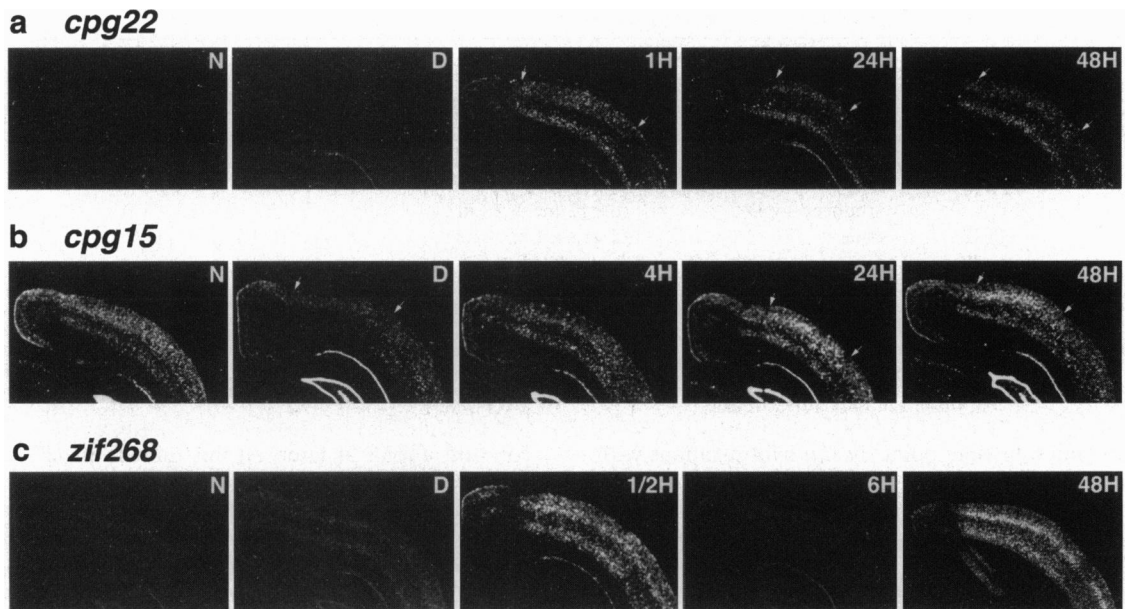


FIG. 1. Time course of *cpg22* (a), *cpg15* (b), and *zif268* (c) induction by light. *In situ* hybridizations of the indicated clones to rat brains sectioned coronally through visual cortex are shown. Animals were placed in the dark for 2 weeks and then exposed to constant light for increased periods before euthanasia: 1/2 hr (1/2H), 1 hr (1H), 2 hr, 4 hr (4H), 6 hr (6H), 12 hr, 24 hr (24H), and 48 hr (48H). Control sections for all probes were from normal untreated rat brains (N) and from brains of rats after 2 weeks in constant darkness (D). Selected times of light exposure representative of the temporal expression pattern of each clone are shown. Arrows mark regions where changes in expression can be seen in response to light.

tains a stretch of 14 amino acids, of which 11 are identical to a similar-sized stretch in dystrophin (Fig. 3d). This analysis strongly suggests that CPG2 is a "minidystrophin-like" protein similar in structure to dystrophin but approximately one-third

the size. The predicted CPG15 protein contains a putative signal sequence and thus may enter the secretory pathway (data not shown). In our screen for CPGs that may be relevant to cortical plasticity, the same 29 "cortical CPGs" that were

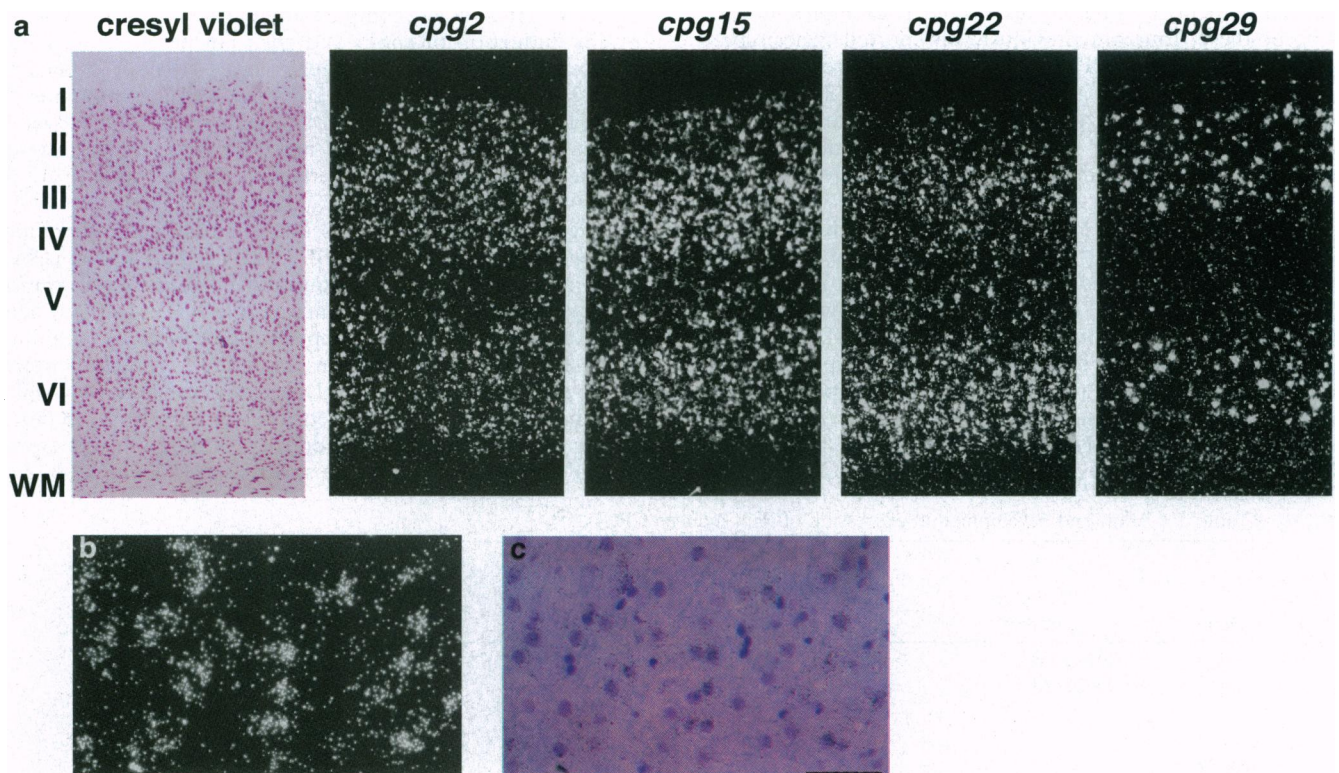


FIG. 2. Layer localization of CPG induction by light in rat visual cortex. (a) On the left is a bright-field micrograph of a Nissl-stained coronal section through visual cortex of a rat exposed to light for 24 hr after dark adaptation. Aligned with this section are dark-field micrographs of *in situ* hybridizations to nearby sections from the same brain, for all the four new CPGs. All sections are 10 μ m thick and located within 10–100 μ m of each other. Layers are designated according to Nissl staining at left. WM, white matter. (b) Dark-field micrograph of cells in layers VI expressing *cpg22*. (c) Bright-field photomicrograph of the same field seen in b, showing autoradiographic silver grains clustered over individual cells. (Bar = 50 μ m b and c.)

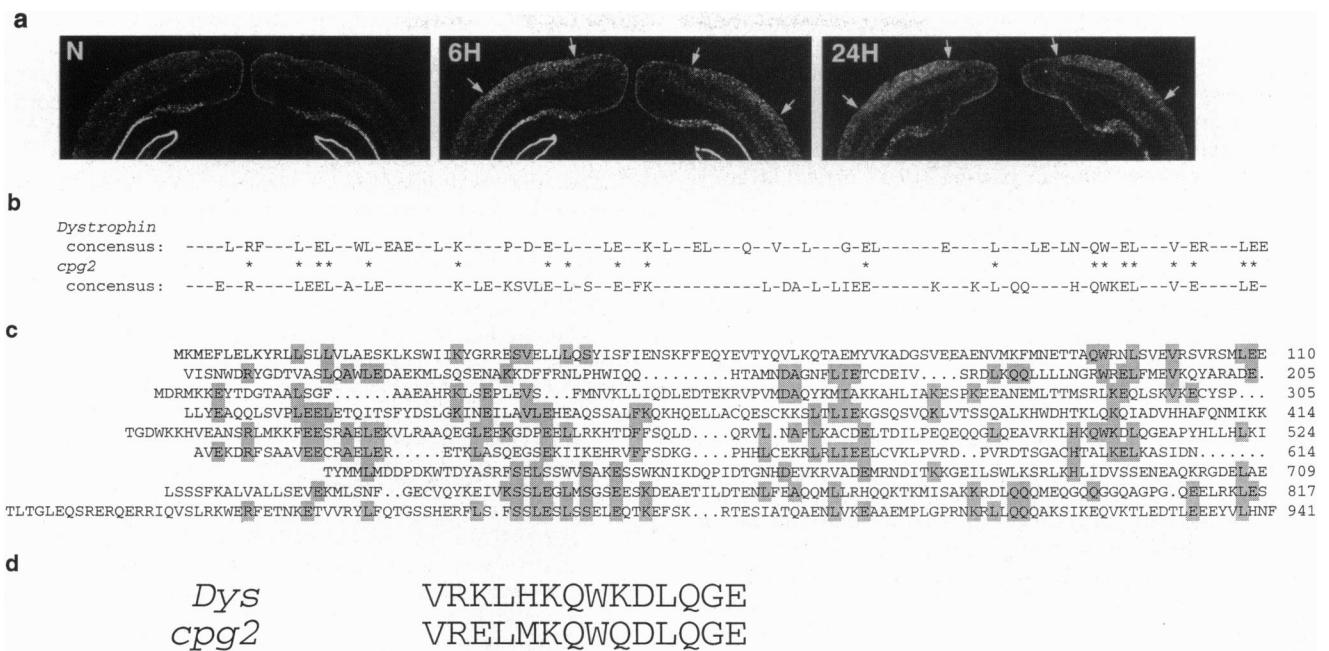


FIG. 3. CPG2 induction by light, predicted protein sequence, and homology to dystrophin. (a) *In situ* hybridizations of *cpg2* to coronal sections through visual cortex from normal untreated rat brain (N) and from rat 6 hr after light exposure (6H) or 24 hr after light exposure (24H). (b) CPG2 protein sequence, deduced from sequencing the full-length *cpg2* cDNA on both strands. The bottom line of *b* shows a consensus that appears in at least three of the CPG2 repeats (shaded in *c*) and is compared to the similarly sized dystrophin repeat (top line of *b*). CPG2 has 15/33 amino acids in positions similar to those of the 41-amino acid dystrophin repeat. (d) An additional 14-amino acid stretch in CPG2 that is homologous to a similar stretch in dystrophin.

tested for light induction in the adult neocortex were also analyzed by *in situ* hybridization for expression in the postnatally developing cortex and compared to their expression in normal adult rat cortex. Twenty-one (75%) of the "cortical CPGs" were found to be transiently expressed in the developing cortex between postnatal days 1 and 21 at levels significantly higher than in normal adult rat cortex. Interestingly, all six light-induced cortical CPGs are included in this group (Table 2). Examples can be seen in Fig. 4: very weak *cpg2* expression can be detected in the cortex 1 day postnatal, increasing gradually and peaking at 10 days postnatal (Fig. 4 Left). *cpg15* expression gradually increases in piriform and entorhinal cortex from 1 day to 14 days postnatal (Fig. 4 Right). Neocortical expression of *cpg15* starts at 10 days postnatal, barely visible at the dorsal midline of the brain (Fig. 4 Right, marked by arrow). Neocortical *cpg15* expression shows robust peak levels at 14 and 21 days postnatal and decreases again in the adult. Interestingly, *cpg15* expression in the lateral geniculate nuclei and somatosensory thalamus is very high in the first few postnatal days and gradually decreases with age (Fig. 4 Right).

DISCUSSION

Characterization of CPG light-induction in adult visual cortex, reveals several unifying features. Although the onset and time course of induction by light vary among the different CPGs, induction of all the visually responsive CPGs is maintained for at least 48 hr after the onset of light exposure. As reported earlier, *zif268*, the only gene shown to date to be induced by light in visual cortex (22), indeed shows an immediate and transient induction. However, we found that light induction of *zif268* is biphasic. The early phase, triggered within half an hour of light exposure and lasting for up to 2 hr, is followed by a second expression phase that begins ≈10 hr later. The prolonged elevation of CPG expression, rather than a transient short-term response, suggests a role for these light-induced CPGs in producing long-term, activity-dependent changes.

In addition to their persistent induction, a second feature common to all four previously unreported, light-responsive

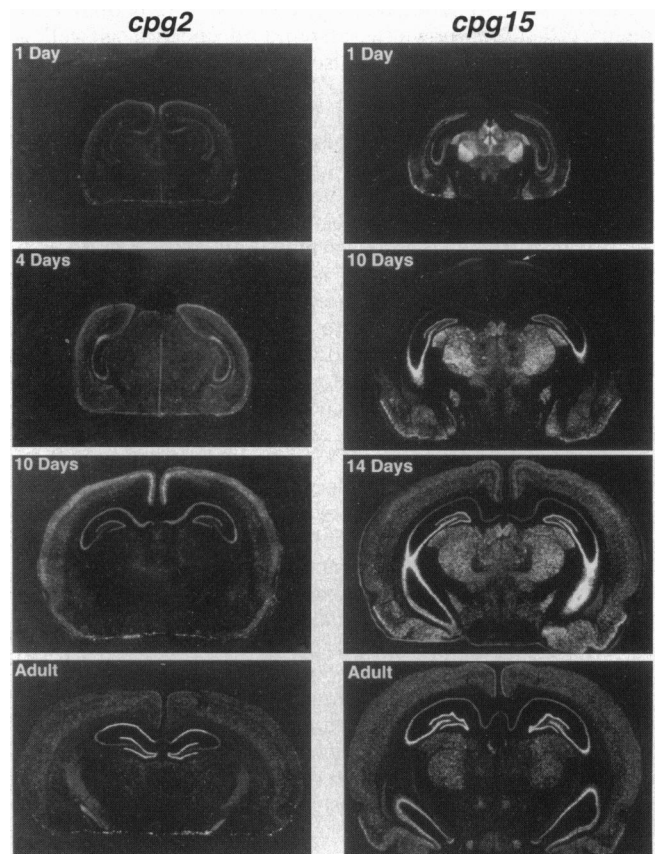


FIG. 4. Postnatal developmental expression of *cpg2* (Left) and *cpg15* (Right). *In situ* hybridizations of *cpg2* or *cpg15* to coronal sections from rat brains at the postnatal ages indicated.

CPGs is their induction in cortical layers II–III and VI. Light-induced gene expression in these layers of visual cortex within 24–48 hr after visual stimulation correlates with the electrophysiologically mapped shift in receptive fields seen in the corresponding supragranular and infragranular layers of rat somatosensory cortex 24 hr after manipulation of afferent activity by “whisker pairing” (9). Cortical layers II and III of adult cat striate cortex were also shown by anatomical methods to undergo structural changes in the form of axonal sprouting during the functional reorganization after focal retinal lesions (8).

Notably, none of the six CPGs exhibited light-induction in the lateral geniculate nucleus of the thalamus (data not shown). This result is consistent with observations that in the adult cat visual system, functional reorganization is confined to the cortex (7).

The predicted CPG2 protein contains features indicative of a *bona fide* structural protein, similar in structure to dystrophin, but approximately one-third the size. Dystrophin-associated glycoproteins have been shown to be involved in agrin-induced receptor clustering at the neuromuscular junction (25, 26). Dystrophin is also localized in postsynaptic densities in the adult brain (27). As *cpg2* is expressed only in the central nervous system (data not shown), CPG2 may be a structural protein with functions related to those of dystrophin in the central nervous system. The predicted CPG15 protein contains a putative signal sequence and thus may enter the secretory pathway (data not shown). Many secreted neuropeptides and trophic factors are responsive to changes in levels of neural activity (28–30). Brain-derived neurotrophic factor, in particular, is regulated (although not induced) by light (31) and has also been implicated in activity-dependent, synaptic reorganization during visual system development (32). The light-induced CPGs therefore encode not only regulatory immediate early genes (20, 21), known to respond to specific as well as nonspecific stimuli (33), but also genes that predict structural and secretory-pathway-directed proteins.

During postnatal cortical development both activity-dependent and activity-independent processes influence final connectivity patterns (15–17, 34). All six light-induced cortical CPGs were found to be transiently expressed in the developing cortex between postnatal days 1 and 21, at levels significantly higher than in normal adult rat cortex. Expression of these CPGs, therefore, is modulated by changes in the level of neural activity regulated by light in the adult cortex and is also regulated during postnatal cortical development. It is not yet known whether during postnatal development, induction of cortical CPGs is activity-dependent or dependent on other environmental cues. However, activation of the same set of genes in the adult and during development under circumstances where synaptic reorganization is likely to take place may hint that similar molecular mechanisms are involved.

Sprouting and synaptogenesis have been observed in a variety of adult mammalian central nervous system structures as a result of stimuli that can produce activity-dependent plasticity (35–38). Recently, axonal sprouting has also been demonstrated during functional reorganization in adult cat striate cortex (8). Structural changes that include axonal sprouting and synaptogenesis would necessitate synthesis of new proteins and, therefore, involve gene induction.

CPGs were originally cloned as induced by kainate in the hippocampus (18), where structural activity-induced changes are known to occur (36–38). Here we present evidence that six CPGs (four of them previously unreported) are genes also induced in the adult by a visual stimulus in cortical layers shown anatomically and physiologically to undergo adaptive cortical plasticity (8, 9). The persistence of CPG induction by light suggests CPG participation in long-term, rather than transient, changes. These light-induced CPGs predict proteins

that are secreted (CPG15) and structural (CPG2) by nature. All this taken together suggests that gene expression induced in adult cortex by physiological stimuli could mediate structural changes that may underlie mechanisms of adult cortical reorganization.

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