Glioma is a group of diseases that leads to irreversible damage to the optic nerve and eventual vision loss. Most of these diseases are characterized by elevated intraocular pressure (IOP). In the primate model of glioma, introduced by Gaasterland and Kupfer, 1 scarification of the trabecular meshwork by an argon laser causes blockage of aqueous humor outflow and leads to elevated IOP. Experimental chronic glioma in the monkey mimics human glioma in optic disc changes and in the pattern of ganglion cell loss.2-4

The loss of ganglion cells due to elevated IOP causes degenerative morphologic changes and an eventual loss of neurons within the central nervous system (CNS). The lateral geniculate nucleus (LGN), the principal thalamic recipient zone of the retina, demonstrates a decrease in metabolic activity, 5 a change in the size distribution and density of cells, 6 and a decrease in the overall number of immunoreactive relay neurons 7 after unilateral elevation of IOP. After 1 year of elevated IOP, further changes occur in the metabolic activity of neurons in the primary visual cortex (VI), the main cortical target of the LGN. 8,9 Taken together, these results suggest that damage to the retinal ganglion cells due to elevated IOP causes degenerative changes in the LGN and primary visual cortex in the adult primate brain. However, electrophysiological studies of cortical neurons in the visual system and other sensory systems have shown that degenerative changes in the CNS are not the only consequence of a loss of sensory inputs. For example, topographical reorganization of sensory maps and an expansion of the cortical representation of the intact neighboring sensory surfaces were first demonstrated in the somatosensory cortex after experimental peripheral denervation and later in the visual system after induction of focal retinal laser lesions. 10-13 More recently, the tonotopic representation in the primary auditory cortex (A1) of patients with high-frequency cochlear hearing loss was also shown to undergo reorganization. 14 These examples suggest that cortical neurons have the capacity for activity-driven synaptic strength changes that may allow them to regain function after peripheral sensory loss. The functional reorganization observed in the cortex of these adult animals after peripheral sensory loss is notable, because less is known about cortical plasticity (the ability of neurons to modify their synaptic responsivity) in the adult brain than about that in the developing brain. 15

Given that elevated IOP results in a peripheral sensory loss, we wondered whether it would induce plastic changes in the visual cortex of the adult primate brain. In this study, we focused on several molecular correlates associated with synaptic changes in the primary visual cortex. Earlier studies using more severe forms of visual deprivation, such as monocular deprivation by lid suture, intraocular injection of tetrodotoxin (TTX), or enucleation, showed that decreases in the metabolic drive of cortical neurons lead to a concomitant redistribution of several neurochemicals associated with synaptogenesis and is implicated in cortical plasticity in the adult visual system. 16-21 Because the changes to the visual system after elevated IOP are more subtle and gradual than those resulting from enucleation, TTX injections, or even lid suture, the present study focused on the early (<1 year) time course of metabolic and neurochemical changes that take place at the level of the primary visual cortex. Using immunohistochemical
methods, we found a redistribution of synaptophysin (SYN), growth-associated protein-43 (GAP-43), γ-aminobutyric acid/benzodiazepine (GABAa) receptor protein, and calcium-dependent protein kinase II α subunit (CAMKIIα). These proteins play an important role in synaptogenesis, axonal sprouting, and the regulation of neuronal activity, and thus their redistribution may underlie functional reorganization in the adult visual cortex in response to the damage to retinal ganglion cells caused by elevated IOP in a primate model of glaucoma.

**MATERIALS AND METHODS**

**Subjects and Procedures**

All studies were performed according to the guidelines of the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research. Ten monkeys (seven rhesus, *Macaca mulatta*; three cynomolgus, *Macaca fascicularis*) of both sexes were used (Table 1). In seven monkeys, chronic elevation of IOP for 2, 4, or 7 months was induced by laser scarification of the trabecular meshwork. One monkey underwent unilateral laser ablation of the trabecular meshwork, but its IOP remained relatively normal, and thus that monkey served as the sham-operation control. Another two animals did not undergo the laser procedure and served as the normal control.

Anesthesia for the laser procedure was ketamine (10 mg/kg, intramuscularly [IM]) plus acepromazine (0.2–1 mg/kg, IM). Frequently, the animals also received IM methohexitol anesthesia, if photography and other imaging were performed immediately before laser treatment. A standard clinical argon laser and slit lamp delivery system was used to produce a series of focal lesions to the trabecular meshwork (75–250 spots, 50 μm spot diameter, 1–1.5 W, 0.5 seconds duration). In all animals IOP was measured under ketamine anesthesia with a minified Goldmann (Haag-Streit, Koniz, Switzerland) applanation tonometer, occasionally backed up by measurements with a handheld tonometer (Tono-pen XL; Mentor O&O, Norwell, MA) if corneal edema, neovascularization, or head and eye movements in animals under ketamine anesthesia prevented readings with the Goldmann. The IOP was measured before application of the laser and weekly thereafter. If IOP was not consistently higher than 30 to 40 mm Hg, additional laser treatments were performed until stable ocular hypertension was achieved. If IOP-lowering therapy was implemented, the IOP was checked more frequently until it stabilized, and then the weekly schedule for checking IOP was resumed. IOP was checked with the monkey lying prone in a head holder. The opposite eye served as a normal control. The IOP for normal monkeys was averaged from measurements taken before their use in other experiments over the course of several months. One normal monkey was used in fluorophotometry and IOP experiments after topical or intravitreal injections. That monkey was then used in 15 outflow facility experiments in which the anterior chamber was cannulated with needles. However, all slit lamp examinations and IOPs were normal before the monkey was included in these experiments. Finally, the same monkey was used in intravitreal herpes simplex virus (HSV) viral vector studies 3 days before death, at which time the cornea became cloudy. The other normal monkey was used only in topical drop-transscleral injection IOP and pupil experiments, in which IOP was not elevated. The sham-operation animal underwent one laser application consisting of 60 burns over a 270° radius, which resulted in no IOP elevation during the 128 days after the laser treatment.

Elevated IOP levels in this study ranged from 30 to 66 mm Hg. If IOP was higher than desired or if there were signs of discomfort, the monkeys were treated topically once or twice daily with a single drop of one or more of the following until the desired IOP was achieved: 0.5% timolol maleate in gel-forming vehicle (Timoptic-XE; Merck & Co., West Point, PA), 0.2% brimonidine tartrate (Alphagan; Allergan, Irvine, CA), 2% dorzolamide hydrochloride (Trusopt; Merck), or PGF2α-isopropylster (2 μg in 5 μL saline, donated by Pharmacia Corp., Peapack, NJ). If necessary, acetazolamide (5 mg/kg, IM; Ben Venue Laboratories, Bedford, OH) was also administered once or twice daily. After the designated periods of IOP elevation, anesthesia was induced with IM ketamine (10 mg/kg), followed by deep pentobarbital anesthesia (35 mg/kg IM or 15 mg/kg intravenously). The chest was opened and each monkey perfused intracardially with 750 mL phosphate-buffered saline (PBS), followed by 1 L 4% paraformaldehyde in PBS followed by 200 to 300 mL PBS.

**Tissue Preparation**

Brain tissue was postfixed in 4% paraformaldehyde for 24 to 48 hours. The primary visual cortex was blocked and flattened. Visual cortex representing the central foveal visual fields as well as those representing the peripheral visual fields were processed. Flattened blocks were cryoprotected in 20% sucrose solution made in phosphate buffer (PB). The frontal cortex was coronally blocked at stereotaxic coordinates between A25.0 and A35.0. All tissue blocks were stored frozen at −80°C. Tangential sections through the full thickness of visual cortex or coronal sections of frontal cortex were cut at 50 μm on a cryostate. Every third section was reacted for cytochrome oxidase (CO) or immunohistochemically for GAP-43, SYN, GABA (β2/3 subunit), or CAMKIIα.

**Cytochrome Oxidase.** CO is a mitochondrial enzyme often used as a metabolic activity marker in the visual cortex. The CO reaction used in this study was adapted from other studies. First, 20 mg of diaminobenzidine (DAB; Sigma-Aldrich Co., St. Louis, MO) was dissolved in 50 mL of distilled water. Once the DAB had dissolved, 50 mL (0.1 M) PB, 2 g sucrose, 30 mg cytochrome c (Sigma-Aldrich Co.), and 20 mg catalase (Sigma-Aldrich Co.) derived from bovine heart were added to the DAB solution. Then, 5 mL of (1%) nickel ammonium sulfate was added in drops followed by approximately 1 mL of (1%) cobalt chloride until the solution appeared slightly opaque. Sections were placed in 12-well plates filled with 1.5 mL of the CO solution and incubated at 40°C for 3 to 6 hours. When the reaction was completed, the sections were washed three times for 5 minutes each in PB. CO activity is reduced in layers 2/3 and 4C of the visual cortex after deprivation. The CO activity is reduced in layers 2/3 and 4C of the visual cortex after deprivation. The CO activity is reduced in layers 2/3 and 4C of the visual cortex after deprivation.
left hemisphere were reacted histochemically for CO and immunohistochemically for GAP-43 and the GABAα receptor (β chain). Sections from the right hemisphere were reacted histochemically for CO and immunohistochemically for SYN and CAMKIIα. All immunohistochemistry was performed by reacting pairs of sections from visual and frontal cortex simultaneously.

Monoclonal antibody against GAP-43 was obtained from the GAP-7B10 hybridoma produced by the fusion of mouse myeloma cells and splenocytes from BALB/c mice immunized with HPLC-purified GAP-43 from neonatal rat forebrain membranes (Sigma-Aldrich Co.). It exhibits a wide interspecies cross-reactivity and was used to localize both phosphorylated and nonphosphorylated forms of GAP-43 in the primary visual cortex.

Monoclonal anti-SYN (Sigma-Aldrich Co.) was obtained from a hybridoma produced by mouse myeloma cells and splenocytes from an immunized mouse. A synaptosome preparation from rat retina was used to react with the β chain of the GABAα receptor in the primate brain. This antibody was obtained by immunizing BALB/c mice with a highly purified GABA-benzodiazepine receptor preparation from bovine brain. It can cross-react with human GABAα with the same subunit specificity as in bovine brain. The β-subunit is present in all brain areas of human cerebral cortex.

A monoclonal antibody to the α subunit of Ca2+/calmodulin-dependent protein kinase type II (CAMKIIα) derived from mouse-mouse hybrid cells (clone 6g9) was used (Roche Molecular Biochemicals, Indianapolis, IN). The antibody was obtained by immunizing BALB/c mice with purified type II CAM protein kinase from the synaptic vesicles isolated from rat brain. Lymphocytes from the immunized mouse spleen were fused with mouse myeloma cells of the cell line NS1/SP2. Anti-CAMKIIα detects a 56kDa protein and reacts specifically with the α-subunit in all mammalian cells.

For GAP-43, SYN, and the GABAα receptor, sections of primary visual cortex were incubated in 5% to 10% normal horse serum (NHS) for 1 hour to block nonspecific binding. Sections were then washed three times for 5 minutes each in PB. GAP-43 and SYN antibodies were used at a 1:2000 dilution in 3% NHS in 0.1% Triton X-100 (TX100) made in PB. Antibody against GABAα receptor was used at a 1:200 dilution in 5% NHS in 0.1% TX100. For CAMKIIα immunoreactivity, sections were preincubated in 0.2% TX100 for 15 minutes, according to the manufacturer’s protocol, before incubation in a 1:1000 dilution in 5% NHS in 0.1% TX100. All immunostained sections were agitated and incubated at 4°C in primary antibody for 36 to 48 hours. Sections were washed three times for 5 minutes each in 0.1% TX100 before being incubated for 2 hours at room temperature in 0.1% secondary antibody (biotinylated anti-mouse made in horse) in 3% NHS in 0.3% TX100 made in PB. Before incubation with the avidin biotin complex for 1 hour, sections were washed three times for 5 minutes each in PB. Antibodies were visualized by using the glucose oxidase-DAB reaction in which 10 mg of DAB, 40 mg dextrose, and 8 mg ammonium chloride are dissolved in 20 mL of PB. Sections were preincubated in the DAB solution for 10 minutes. Next, glucose oxidase (6 mg glucose oxidase in 50 mL distilled water) was added to the DAB solution at a 1:10 ratio, and the reaction proceeded in the dark for 45 minutes. Sections were then rinsed three times in PB for 5 minutes, mounted onto gelatin-coated slides, air dried, dehydrated through an ascending concentration of alcohols, cleared in xylene, and coverslipped in mounting medium (Permount; Fisher Scientific, Fair Lawn, NJ). Control sections for each marker used the same protocol, except that the primary antibody was omitted.

Optical Measurements

Image Capturing. All CO-stained and immunoreacted sections of layer 4C of the visual cortex were analyzed by densitometry. Video images were captured on a light box (Aristo, Waterbury, CT) with a charge-coupled device camera (model 4915; Cohu, Inc., San Diego, CA) attached to a micro 55-mm lens (Nikkor; Nikon, Melville, NY). The camera was connected to a computer-based analysis system (Macintosh; Apple Computer, Cupertino, CA) with a quick-capture board (model DT-2255; Data Translation, Marlboro, MA). The setting on the light box and F-stop of the lens remained constant throughout at 100 and f16 respectively. The computer image-processing and image-analysis software NIH Image 1.62 (available by ftp from zippy.nih.gov or from http://rsb.info.nih.gov/nih-image; developed by Wayne Rasband, National Institutes of Health, Bethesda, MD) was used to obtain optical density (OD) profiles and to measure periodicity of ocular dominance columns. Raw image intensities were converted to ODs with a standardized curve made with neutral density filters of known transmittance.

Optical Density. For OD measurements in the visual cortex, transects were placed in layer 4C in tangential sections. In the case of glaucomatous eyes in which CO staining demonstrated a series of light (glaucomatous) and dark (normal) ocular dominance bands, OD measurements were made in both the glaucomatous and normal eye bands, and a within-animal (e.g., between ocular dominance bands) comparison obtained. The mean ODs of the glaucomatous and normal eye bands were obtained from 30 transects for each of the four neurochemical markers. The mean was then divided by the mean OD of the frontal cortex of the same animal to obtain a normalized OD for each neurochemical (Figs. 6A–D). For OD measurements in coronal sections of frontal cortex, transects were placed perpendicular to the pial surface spanning all six cortical layers from pial surface to white matter.

Next, a between-animal comparison was performed on the densitometric values obtained by immunostaining in experimental and normal animals to assess whether overall immunoreactivity changes in response to elevated IOP. Tissues from glaucomatous and normal animals were not reacted simultaneously. To control for differences in densitometric values associated with histochemical technique, which may vary from day to day and thus change the background level of immunoreactivity, we normalized densitometric values obtained from the visual cortex with those obtained from frontal cortical tissue (anterior posterior level, +20 mm) of each experimental animal. For each animal, frontal and visual cortical tissues were reacted simultaneously in the same well. We assumed that the levels of the neurochemicals would not vary significantly in the frontal cortex of the experimental animal after elevated IOP, because the frontal cortex is not known to have a direct input from the LGN or any other major visual thalamic center. This assumption was verified for three of the four markers by experiments in which tissue sections from the frontal cortex of a normal animal and the frontal cortex of an experimental animal were reacted simultaneously (Figs. 6E).

Statistical Analysis

Differences in ODs of glaucomatous and normal eye columns and differences between ODs in tissue sections from glaucomatous and normal animals were assessed by using repeated-measures within-factor analysis of variance (ANOVA) followed by post hoc comparisons. All calculations were performed with statistical analysis software (StatView 4.02; Abacus Concepts, Inc., Berkeley, CA). Mean differences of P ≥ 0.001 were considered significant.

RESULTS

Sham-Operation Animal

Tissues from the animal that underwent a sham IOP-elevating procedure animal were processed to determine whether the surgical effects themselves and not the elevated IOP contributed to the results. This monkey underwent laser scarification, but maintained a normal IOP of 20 ± 3 mm Hg in each eye. Tissue processing revealed a normal pattern of CO staining throughout the primary visual cortex. The CO blobs in layers 2/3 were uniform in size and density, and those associated with the ocular dominance columns of the laser-treated eye were
indistinguishable from those associated with the normal fellow eye (Fig. 1A). The qualitatively normal pattern of staining in the sham-operation animal was particularly evident in comparison to the abnormal pattern of CO blobs observed at 4 (Fig. 1E) and 7 (Fig. 1G) months of elevated IOP. CO staining of layer 4C, the major LGN input layer, demonstrated a normal staining pattern that was uniform with non-fluctuating OD (Fig. 1B). Immuno-histochemistry did not reveal any fluctuation in staining for the four neurochemicals studied (Figs. 2A, 2B, 3A, 3B, 4A, 4B, 5A, 5B).

Immunoreactivity of Frontal Cortical Tissue in Normal and Experimental Animals

An experiment was undertaken to determine whether elevated IOP affects the immunoreactivity of tissue from the frontal cortex, a cortical area that does not receive a direct projection from subcortical visual centers such as the LGN or a direct projection from V1. If the immunoreactivity of the frontal cortex is independent of visual deprivation by elevated IOP, the use of frontal cortical tissue to standardize immunoreactivity for between-animal comparison seems valid. For this experiment, sections of the frontal cortex of experimental animals (n = 3) with elevated IOP and normal animals (n = 2) were reacted simultaneously with each of the four antibodies. The mean OD for immunoreactivity in the frontal cortex is shown in Figure 6E. Results for GAP-43, GABAa receptor, and SYN immunoreactivity in the frontal cortex were not significant, thus validating the use of frontal cortical tissue to standardize immunoreactivity for the between-animal comparison for GAP-43, GABAa receptor, and SYN. Unexpectedly, the OD of CAMKIIα immunoreactivity in the frontal cortex of experimental animals was significantly reduced compared with levels in normal animals. Although it is difficult to interpret the significance, the finding was confirmed by repeating the experimental procedures to ensure there were no methodological artifacts. Furthermore, the results

FIGURE 1. CO histochemistry in layers 2/3 (A, C, E, G) and layer 4C (B, D, F, H). CO was reacted on tangential sections of visual cortex from an animal that underwent a sham operation (A, B) or animals with elevated IOP for 2 (C, D), 4 (E, F), or 7 (G, H) months. Serial tangential sections were aligned, with the cross-sections of blood vessels used as landmarks (arrows). The CO blob pattern in the sham-operation animal (A) and the animals with 2 months of elevated IOP (C) differed from the pattern in the animals with 4 (E) and 7 (G) months of elevated IOP. After 4 and 7 months of elevated IOP, rows of pale, shrunken CO blobs alternated with rows of more robust CO blobs. In layer 4C, alternating dark and light CO-stained ocular dominance bands were seen beginning at 2 months (D) and persisted after 4 (F) and 7 (H) months of elevated IOP.

FIGURE 2. CO histochemistry and GAP-43 immunoreactivity in tangential sections through layer 4C are shown in a sham-operation animal (A, B) and in animals with 2 (C, D), 4 (E, F), and 7 (G, H) months of elevated IOP. CO histochemistry (A) and GAP-43 immunoreactivity (B) resulted in uniform staining in the sham-operation animal. Ocular dominance bands and GAP-43-immunoreactive bands were present in the animals with 2 (C, D), 4 (E, F), and 7 (G, H) months of elevated IOP. The dark immunoreactive bands for GAP-43 were aligned with the normal eye's ocular dominance bands. Serial adjacent sections were aligned, with cross-sections of blood vessels used as landmarks (arrows).
were consistent among all experimental animals at all time points. Because the CAMKII/H9251 immunoreactivity in the frontal cortex of the experimental animals was significantly reduced compared with levels in normal animals, between-animal comparisons were not made for CAMKII/H9251 in this study.

Experimental Animals

Cytochrome Oxidase. After 2 months of elevated IOP, the pattern of CO blob staining was qualitatively normal in layers 2/3 (Fig. 1C). In contrast, the pattern of CO staining in layer 4C demonstrated an abnormal CO pattern in which lightly stained CO bands, representing the ocular dominance bands associated with the laser-treated eye interdigitated with the darkly stained CO bands representing the ocular dominance bands associated with the normal fellow eye (Fig. 1D).

After 4 and 7 months of elevated IOP, the pattern of CO staining in layers 2/3 demonstrated a deprivation effect. Rows of shrunken CO blobs alternated with rows of larger interconnected CO blobs (Figs. 1E, 1G). Alignment of tangential sections including the cross-sections of major blood vessels demonstrated that the shrunken CO blobs were located in the centers of the light CO bands in layer 4C (Figs. 1E–H). Alternating dark and light CO bands were found in layer 4C of all visual field representations at all three deprivation periods (2, 4, and 7 months of elevated IOP; Figs. 1D, 1F, 1H).

Growth-Associated Protein-43. After 2, 4, and 7 months of elevated IOP, differential GAP-43 immunostaining was observed. At all time points, the pattern of light and dark immunostained GAP-43 bands (Figs. 2D, 2F, 2H) coaligned with the light and dark CO-stained bands, respectively (Figs. 2C, 2E, 2G). Thus, GAP-43 immunoreactivity was strongest in the normal eye bands and weakest in the glaucomatous eye bands.
However, despite the lowered OD of GAP-43 immunoreactivity in the glaucomatous eye bands, when compared with the normal eye bands, both were significantly greater than the OD of GAP-43 immunoreactivity in layer 4C of the visual cortex of normal animals (Fig. 6A).

**α-Subunit of the Receptor for GABAA.** Immunostaining against the GABAA receptor revealed a pattern of alternating light and dark bands at all three deprivation periods, similar to GAP-43 immunostaining. The light GABAA receptor bands corresponded with the glaucomatous eye bands and the dark GABAA receptor bands corresponded with the normal eye bands (Figs. 3C–G). However, unlike the findings for GAP-43, both glaucomatous and normal eye bands demonstrated lowered ODs for GABAA receptor immunoreactivity than that seen in the visual cortex of normal animals (Fig. 6C). Thus, in response to elevated IOP, expressed levels of the GABAA receptor subunit were downregulated throughout layer 4C of area V1.

**Calcium-Dependent Protein Kinase IIα.** After 2 months of elevated IOP, immunostaining against CAMKIIα revealed a pattern of alternating light and dark bands, but with a reverse pattern to that seen for the other markers. Unlike GAP-43 and GABAA receptor immunostaining, the lightly immunoreactive CAMKIIα bands corresponded with the normal eye bands and the darkly immunoreactive CAMKIIα bands corresponded with the glaucomatous eye bands (Figs. 4C, 4D). This reverse pattern of immunostaining for CAMKIIα was present at 2 (Figs. 4C, 4D), 4 (Figs. 4E, 4F), and 7 (Figs. 4G, 4H) months of elevated IOP. The OD of CAMKIIα immunoreactivity was significantly greater in the glaucomatous eye bands than in the normal eye bands (Fig. 6D).

**Synaptophysin.** The pattern of immunostaining for SYN was unlike those of the other neurochemical markers in the study. There was no apparent pattern of light and dark immunoreactive bands after 2 (Fig. 5D) or 7 (Fig. 5H) months of elevated IOP, even though light and dark CO-stained bands were visible at both these time points (Figs. 5C, 5E, 5G). A pattern of alternating light and dark immunoreactive bands was visible in layer 4C only at 4 months of elevated IOP. The dark SYN bands coaligned with the dark CO bands associated with the normal eye, and the light SYN bands coaligned with the light CO bands associated with the glaucomatous eye (Figs. 5E, 5F). The OD of SYN immunoreactivity of the normal eye bands in glaucomatous animals was significantly greater than ODs of layer 4C in the visual cortex of a normal animal, but the ODs for the glaucomatous eye bands were not (Fig. 6B).

**DISCUSSION**

This study shows that experimental unilateral glaucoma alters the spatial and temporal distribution of several neurochemicals associated with cortical plasticity in V1 of the primate. When we compared CO activity between the normal and glaucomatous eye’s ocular dominance bands in layer 4C, CO activity in the glaucomatous eye’s bands was reduced at all time points, consistent with earlier studies that reported a loss of CO activity after other forms of unilateral visual deprivation. Visual deprivation by monocular enucleation, lid suture, and retinal intraocular blockade has been shown to lead to a downregulation of CO in the affected ocular dominance bands of adult monkeys.19,27,30,31–34

**Within-Animal Comparisons**

**Differential Regulation of Neurochemicals in Ocular Dominance Bands.** The observed pattern of CO staining in layer 4C demonstrated a pattern in which lightly stained CO bands, representing the ocular dominance bands associated with the laser-treated eye, interdigitated with the darkly stained CO bands, representing the ocular dominance bands associated with the normal, fellow eye and provided landmarks to assess the effect of elevated IOP on the immunohistochemical levels of GAP-43, SYN, GABAA, and CAMKIIα. We compared the OD of immunoreacted tissues between the glaucomatous and normal eye’s ocular dominance bands within a given experimental animal. There was a detectable change in OD between glaucomatous and normal eye bands for all four neurochemicals, but the spatial patterns and time course differed. For GABAA receptor protein (Fig. 6C) and GAP-43 (Fig. 6A) immunoreactivity, the OD of the ocular dominance bands associated with the glaucomatous eye was significantly lower than that of the normal eye bands at all three time points. The OD of SYN immunoreactivity was greater in the normal compared with the glaucomatous eye’s ocular dominance bands, consistent with the spatial pattern for GABA and GAP-43, but unlike those proteins, SYN changes were observed only at the 4-month time point (Fig. 6B). The within-animal comparisons...
of GAP-43, GABAa receptor, and SYN immunoreactivity suggest that these proteins are regulated in an activity-dependent manner and thus are more strongly expressed in the normal eye.

CAMKIIα immunoreactivity showed a converse pattern. The OD of the glaucomatous eye bands was greater than the normal eye bands, suggesting that CAMKIIα is upregulated in the glaucomatous bands and/or downregulated in the normal bands (Fig. 6D). Recent studies have shown that CAMKIIα interacts with the N-methyl-D-aspartate (NMDA) receptor and is implicated in the synaptic plasticity underlying learning and memory. That its levels are relatively greater in the glaucomatous eye bands suggests that a loss of metabolic activity of the retinal ganglion cells due to the elevated IOP may initiate synaptic plasticity changes and subsequent cortical reorganization within the glaucomatous eye bands first.

**Time Course of Neurochemical Changes.** The relative changes in immunoreactivity for three of the four markers persisted for the duration of the elevated IOP (Figs. 2, 3, 4). Whether changes in protein levels of GAP-43, GABAa, and CAMKIIα will affect the function of cortical neurons is not known in this model. However, electrophysiological studies of feline visual cortex after focal retinal laser lesions have shown that cortical neurons silenced by loss of retinal ganglion cell activity recover responsivity over a time course of months, similar to the time course of changes in neurochemical distribution in the current study.

For SYN, detectable changes in the OD were present only at the 4-month time point (Fig. 5). It is not known why SYN levels did not change in response to elevated IOP at the 2- and 7-month time points. That no effect was seen until 4 months of elevated IOP suggests that the activity-dependent regulation of SYN occurs more slowly (i.e., >2 months) after elevated IOP. The absence of change in SYN immunoreactivity between glaucomatous and normal eye bands after 7 months of elevated IOP suggests that there may be a leveling off, leading to a diminution of the differential staining pattern at the 7-month time point. This study, however, cannot rule out the possibility that SYN levels changed simultaneously in normal and glaucomatous eye bands, which would result in no relative change and which would also be consistent with the findings after 2 and 7 months of elevated IOP.
Between-Animal Comparisons

Growth Associated Protein-43. Overall levels of GAP-43 increased in experimental animals when compared with levels in the visual cortex of normal animals (Fig. 6A). An overall increase in GAP-43 protein density in both the glaucomatous and normal eye’s ocular dominance bands in experimental animals, at all time points, suggests that axonal growth occurs in both the glaucomatous and normal eye bands. These results are consistent with earlier studies in which deprivation by focal retinal lesions produced local sprouting of axon collaterals into neighboring, intact visual cortical zones.\(^1\)\(^2\)\(^3\)\(^4\)\(^5\)\(^6\)\(^7\)\(^8\) The expression of GAP-43 protein is found in the axon terminals of neurons\(^9\)\(^10\) and also in the neuropil surrounding the neurons.\(^1\)\(^2\)\(^3\)\(^4\) GAP-43 is also associated with axon elongation,\(^1\)\(^2\)\(^3\)\(^4\)\(^5\)\(^6\)\(^7\)\(^8\) and several studies have shown that expression of GAP-43 increases after neuronal injury.\(^1\)\(^2\)\(^3\)\(^4\)\(^5\)\(^6\)

GABA\(_a\) Receptor. Overall levels of GABA\(_a\) receptor protein decreased in response to elevated IOP (Fig. 6C), which is consistent with earlier studies that reported a downregulation of GABA\(_a\) receptor after visual deprivation by monocular lid suture and TTX injections.\(^19\)\(^20\)\(^21\) It has been shown that GABA\(_a\) receptors are regulated by visual activity,\(^22\) which accounts for the protein density’s being consistently greater in the visual cortex of normal eyes than in that of glaucomatous eyes (Fig. 6C). Because the GABA\(_a\) receptor is involved in inhibitory transmission throughout the CNS,\(^19\)\(^20\)\(^21\) a decrease in immunoreactivity suggests a decrease in inhibitory transmission in response to elevated IOP. It may be that a decrease in inhibition, causing a disinhibition, is necessary for axonal sprouting and the upregulation of GAP-43 in adult V1. It is also possible that a decrease in GABA\(_a\) may reflect an activity-dependent gain-control mechanism whereby cortical neurons downregulate inhibitory transmission in response to decreased excitatory drive.

Synaptophysin. SYN immunoreactivity resulted in a banded pattern of staining only at 4 months of elevated IOP. The OD level in the normal eye bands was significantly greater than in normal visual cortex, but the level in the glaucomatous eye bands was not (Fig. 6B). In this study, the regulation of SYN levels appears to lag behind GAP-43 levels. An earlier study reported that changes in GAP-43 immunoreactivity preceded those of SYN immunoreactivity after deafferentation of the dentate gyrus in the hippocampus.\(^24\) It is likely that axonal growth and stability are necessary before SYN levels increase. SYN immunoreactivity correlates with synaptic profile distributions\(^1\)\(^2\) and levels of this marker have been used to quantify presynaptic terminals in the CNS.

Calcium-Dependent Protein Kinase I\(\alpha\). CAMKII\(\alpha\) immunoreactivity was significantly lower in the frontal cortex of glaucomatous eyes than in normal animals. This finding was unexpected, because no known direct projection between V1 and frontal cortex exists and also because there was no effect of elevated IOP on the other neurochemicals studied here. However, because CAMKII\(\alpha\) is a major postsynaptic density protein\(^1\)\(^2\)\(^3\)\(^4\)\(^5\) associated mainly with excitatory synapses,\(^5\) the reduction in CAMKII\(\alpha\) levels in the frontal cortex of glaucomatous eyes suggests that there is an overall reduction in excitatory synaptic function in the frontal cortex. The possibility also exists that other factors may have affected the CAMKII\(\alpha\) levels in this study. More studies are needed to determine the multiple roles of CAMKII\(\alpha\) in the CNS.

This study demonstrates that elevated IOP can modify the expression patterns of GAP-43, GABA\(_a\) receptor, SYN, and CAMKII\(\alpha\) with as little as 2 months of elevated IOP in adult primates. These proteins play an important role in axonal sprouting, synaptogenesis, and the regulation of neuronal activity in the CNS. Thus, although earlier studies focused on the degenerative changes that occur in the CNS in response to elevated IOP, the results in the current study suggest that neurons in the visual cortex regulate the expression of cortical plasticity molecules in response to the initial reduction in metabolic activity of retinal ganglion cells, induced by elevated IOP. Future studies are needed to determine whether the regulation of these molecules can affect the evoked activity of cortical neurons in a primate model of glaucoma.

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