The Projection from V1 to Extrastriate Area 21a: A Second Patchy Efferent Pathway that Colocalizes with the CO Blob Columns in Cat Visual Cortex

The different patchy organizations of neurons projecting from primary visual cortex (area 17) to the various extrastriate areas may contribute to functional differences in the output to each of these areas. The pattern of neurons projecting to extrastriate area 21a was examined using large injections of retrograde tracers and compared to the pattern shown by neurons projecting to the lateral suprasylvian area (LS). Patches of neurons projecting to 21a showed a bimodal laminar distribution, with numerous labeled cells in the upper and lower third of layer 3 bracketing a sparsely labeled central third; LS-projecting neurons were confined to the lower and middle thirds of layer 3. The 21a projecting cells were more tightly clustered in their clustering pattern than the LS projecting cells, i.e. the difference in labeling density between patch and interpatch zones was greater for 21a-projecting cells than for LS-projecting cells. As previously shown for the LS-projecting cells, patches of 21a-projecting cells colocalized with CO blob columns in area 17. Combined with our earlier results, this study shows that the CO blob compartment in area 17 give rise to at least two distinct efferent pathways, one projecting to LS and the other to 21a, and furthermore suggest that each pathway may carry unique information to its extrastriate target.

Introduction
Cat visual cortex has been divided into primary visual cortex and as many as 16 other visually driven areas (Spear, 1991). Each extrastriate area is characterized as having a single representation of visual space (Tusa et al., 1981; Sereno and Allman, 1991), and it is presumed that each subserves a unique role in visual processing. Area 21a contains a very limited representation, restricted primarily to the upper portion of the central 20° of the visual field (Tusa and Palmer, 1980). This extrastriate area is specialized for the detection of the orientation of long, continuous elements such as lines and edges, and thus appears to be important for the processing of form vision (Wimbborne and Henry, 1992; Dreher et al., 1993; Toyama et al., 1994). Electrophysiological studies in another extrastriate area, the lateral suprasylvian (LS) area, suggest that this area is also specialized, but for encoding the motion and direction of moving textures (Blakemore and Zumbroich, 1987; Zumbroich and Blakemore, 1986; von Grünau et al., 1987; Rauschecker, 1988; Takagi et al., 1992; Yin and Greenwood, 1992; Bando et al., 1996; Kim et al., 1997; Mulligan et al., 1997). Other studies using inactivation of LS by cryogenic probes or lesioning have similarly concluded that LS is involved with a motion-processing function (Pasternak et al., 1989; Lomber et al., 1994).

Here, we focus on the efferent neurons in area 17 that project to extrastriate area 21a, which is thought to have physiological properties associated with form processing. We compare the columnar and laminar arrangement of these neurons with those that project to area LS, which is thought to be involved in motion and direction processing. Earlier anatomical studies using small, focal injections of retrograde tracers reported that the projections from area 17 to both 21a and LS arose from neurons in the supragranular layers and were patchy and periodic (Symonds and Rosenquist, 1984a,b). A more recent study (Dreher et al., 1996a) found little difference in projections from area 17 to areas LS and 21a, although differences in the other projections to these two areas were found.

In this study, we found that the pyramidal cells projecting to area 21a were bilaminarily organized, with cells located in the upper and lower tiers of layer 2/3, while the pyramidal cells projecting to LS were located in the middle and lower tiers of layer 2/3. Double-labeling studies reported here revealed that, although the projections from primary visual cortex to LS and 21a have partially overlapping laminar distributions, they arise from separate populations of supragranular pyramidal cells. Although the two populations of efferent neurons were both modularly organized and patchy, they demonstrated quantitative differences in their tightness of clustering. We also compared the distribution of the patches of 21a efferent cells to the cytochrome oxidase (CO) blob columns in alternate serial sections of coronal and flattened preparations of area 17. As for the LS-projecting neurons (Boyd and Matsubara, 1999), CO blob columns have the highest density of 21a-projecting cells with interblob columns having the lowest density of labeled cells, and blob borders demonstrating an intermediate density of labeled cells.

The same set of cortical columns (the CO blob columns) thus give rise to projections to two functionally very different extrastriate areas. Yet, differences in the laminar origins of these two projections suggest that the information carried by each pathway might be only partially overlapping. Thus, like the primate visual system, cat V1 has an anatomical framework that could act to segregate functionally different outputs into the appropriate extrastriate areas (Zeki and Shipp, 1988). An abstract of this work has been published previously (Conway et al., 1996).

Materials and Methods

Surgical Procedures
Data in this paper were obtained from six experiments involving bulk injections of retrograde tracers into area 21a of adult cats of both sexes. Three animals received injections of cholera toxin subunit B conjugated to 7 nm colloidal gold particles (CTX-gold; List Biological, 1% in 0.9% sterile saline) or wheatgerm agglutinin–horseradish peroxidase (WGA-HRP, 1% in 0.9% saline; Sigma) into 21a. Three animals received large injections of FITC or Texas Red labeled dextran-amine (Molecular Probes, 10–15% in distilled water) placed into LS and 21a respectively. There were three other experiments involving bulk injections of retrograde tracers into area 21a that were used for a separate study on the...
geniculocortical afferents to 21a. The cortical labeling from these experiments was consistent with results described below.

Prior to surgery, animals were pronase-treated with a s.c. injection of glycopyrrolate (0.05 mg/kg; Associated Veterinary PurTchase Co., Ltd), i.m. injection of ketamine (20 mg/kg; Associated Veterinary Purchasing Co. Ltd) and diazepam (2 mg/kg; Sabex Inc.). Anesthesia was induced and maintained by trifluorothane (halothane; MTC Pharmaceuticals) inhalation through an endotracheal tube. Body temperature and EKGs were monitored throughout the surgical procedure. Animals were given an i.m. injection of 0.1 g/ml dexamethasone (Austin Laboratories) to reduce brain edema. The site of the incision was injected with 5–5 µl of a long-lasting local anesthetic, 0.25% bupivacaine hydrochloride (Marocene; Winthrop Laboratories). Under sterile surgical conditions, a craniotomy and durotomy were performed over stereotaxic coordinates AP –5 to +6 and ML 7–15 mm (for LS) and AP –7 to +2 and ML 7–15 mm (for 21a).

LS injections were made into the medial bank of the suprasylvian sulcus, beginning just anterior to the genu where it becomes the posterior suprasylvian sulcus, and continuing anteriorly for ~10 mm. The injection pipettes had tip diameters of 10–20 µm and long, narrow tapers and were tilted medially at 30°–45° (depending on AP level) from vertical in order to follow the slope of the sulcus. Ten to fifteen 500–750 µm spaced penetrations were made through the 4–5 mm depth of the sulcus, injection intervals were 1 mm intervals along the injection track. About 10–12 µl of tracer was injected in total in each experiment.

The 21a injections were made in an arc on the posterior portion of the suprasylvian gyrus, just medial to the genu, covering a 5 × 5 mm area. The injection pipettes were tilted perpendicular to the crown of the gyrus in which 21a is located. Multiple injections spaced 500–750 µm apart were made on the surface of this gyrus under visual guidance. Each injection was made at a depth of ~1 mm from the pial surface. A total of 5–8 µl of tracer was injected. At the conclusion of the injections, the craniotomy was packed with saline-soaked gelfoam, the fascia and skin overlying the wound were sutured, and the animal was allowed to recover.

**Histology**

Survival times for experiments involving WGA–HRP and CTX-gold ranged from 1 to 4 days, after which the animals were given an overdose of barbiturate anesthetic and perfused transcardially with 1500 ml phosphate buffer (0.1 M, pH 7.2) with 0.5% sodium nitrite followed by 400 ml of 4% paraformaldehyde in phosphate buffer. The visual cortex from two animals was flattened according to the method illustrated in Figure 1 of Anderson et al. (Anderson et al., 1998), with minor changes in the placement of cuts used to relieve the intrinsic curvature of the cortical mantle. The cortical blocks were left for 16–40 h in 4% paraformaldehyde and 25% sucrose in phosphate buffer between glass plates to flatten. The flattened blocks were sectioned at 50 µm with a freezing microtome. Tissue from one of the animals was blocked stereotaxically and coronal sections of 50 µm thickness were cut. Sections from animals receiving injections of WGA–HRP were reacted using the standard tetramethyl-benzidine (TMB) method (Mesulam, 1978) usually followed with stabilization in chilled ammonium heptamolybdate and cobalt-diaminobenzidine (Horn and Hoffman, 1987). CTX-gold was visualized by silver intensification using the Janssen IntenSE M kit with incubation times ranging from 1 to 2 h.

Sections were reacted for CO using the cobalt-enhanced protocol of Silverman and Toettel (Silverman and Toettel, 1987) modified for free-floating sections or, in later experiments, the following more sensitive protocol using both cobalt and nickel. Earlier studies have found nickel, alone or in combination with cobalt, to be very effective in enhancing CO staining (Crockett et al., 1993; Dyck and Cynader, 1993; Liu et al., 1993). The CO reaction solution we used consisted of 20–25 mg diaminobenzidine, 30 mg cytochrome C, 15 mg catalase and 1 g sucrose dissolved in 100 ml 0.06 M phosphate buffer, pH 7.2, to which was added 3–5 µl of a 1% nickel ammonium sulfate solution and 3–5 µl of a 1% cobalt chloride solution. The presence of catalase in the reaction media helped ensure that the CO reaction did not cross-react with the peroxidase in experiments where WGA–HRP was used as the retrograde tracer. Lack of cross-reactivity between CO and peroxidase was evidenced by the lack of any elevation of CO staining at the location of the WGA–HRP injection sites (not shown). Incubation times ranged from 2 to 4 h. In most cases, sections were divided into two one-in-two series, one of which was reacted for the tracer, and the other was reacted for CO. In other cases, only selected sections from the middle depths of the cortex (layers 3 and 4) were stained for CO.

In those experiments in which we injected fluorescent tracers, animals were killed after a post-injection period of 2 weeks. Perfusion methods were similar to those described above, but with an additional 1000 cm² of fixative. The visual cortex was blocked stereotaxically and 250 µm coronal sections were cut on a vibratome. Fluorescently labeled tissue was imaged using a BioRad MRC 600 confocal microscope employing an argon ion laser (488 and 514 nm). Serial, optical sections were taken and compressed to form a single projected image. Optical sections were digitally processed with a Kalman filter (Woods and Radewen, 1977) to optimize the signal-to-noise ratio.

**Data Analysis**

The procedures used to display labeled cells graphically have been described previously (Boyd and Matsubara, 1994). Briefly, labeling was charted with a computer microscope system consisting of a Nikon Optiphot compound microscope, an x,y coordinate stage encoder, a PC computer running custom software, and a drawing tube focused on the computer monitor. The positions of labeled cells, and of many radially penetrating blood vessels, were recorded, the latter being used as landmarks to align serial tangential sections. Using Igor Pro 3.1 software (Wavemetrics, Inc.) cells from alternate serial tangential sections through the same hemisphere were aligned and displayed together, providing a ‘surface’ view of the labeling pattern with labeling from different laminae collapsed onto a single plane. These data were then binned into 0.1 × 0.1 mm squares and expressed as density of cells/mm² in what we call a ‘two-dimensional histogram’, essentially an image where the value of each pixel corresponded to the number of cells found in that 0.1 × 0.1 mm portion of the image. A Gaussian low-pass filtered version of each set of labeling data was made by centering a 0.25 mm radius convolution kernel on the position of every x,y data point.

A tightness of clustering analysis was performed on cell charts of retrogradely labeled cells in area 17 arising from injections in either 21a or LS by comparing the density of labeling in clusters to the intercluster labeling density. The Gaussian filtered images of the cell charts were low-pass filtered with a radius of 1 mm in Photoshop (Adobe, Inc.) and thresholded into thirds. The densest third of the image was taken to correspond to the cell clusters and the least-dense third was taken as a measure of intercluster density. The density of cells in these two compartments was then calculated using Igor Pro, and a clustering index was calculated by the following algorithm: \((a + b)/(a - b)\) where \(a\) is the density of cells in the densest third of the image, and \(b\) is the density of cells in the least-dense third of the image. With this metric, labeling patterns with no clustering, i.e. spatially random patterns of cells, would give indices near 0, while labeling patterns with all the cells clustered into one-third or less of the cortical area available would give clustering indices of 1.

Positions of labeled cells were compared to the CO blobs in adjacent sections stained for CO. Images of sections stained for CO were obtained with a Cohu CCD camera (4915) and a Data Translation (DT-2255) frame-grabber card using NIH Image software. These images were then superimposed with charted labeling using blood vessels as landmarks.

Two different methods were used to examine the relationship between CO staining and 21a-projecting neurons (Boyd and Casagrande, 1999). A chi-squared analysis was used to determine if the density of cells inside and outside of blobs was significantly different from chance. Positions of blobs and interblobs were determined in a manner similar to the clustering index described above. The CO image was first low-pass filtered to remove low-frequency fluctuations in staining density not related to CO blobs, and then smoothed with a Gaussian convolution. The image was then divided into three equal-sized compartments of blobs, interblobs and blob borders. Using three compartments instead of two was done in recognition of the fact that blob/interblob borders are not sharp and distinct but gradual and fuzzy, showing the need for a blob and interblob method. Related to CO blobs, and then smoothed with a Gaussian convolution. The image was then divided into three equal-sized compartments of blobs, interblobs and blob borders. Using three compartments instead of two was done in recognition of the fact that blob/interblob borders are not sharp and distinct but gradual and fuzzy, showing the need for a blob and interblob method.
The second analysis involved an adaptation of the density recovery profile of Rodieck (Rodieck, 1991). This method was previously adapted to examine the relationship between retrogradely labeled cells and CO staining (Lia and Olavarria, 1996) by comparing the average density of labeled cells at different distances from CO blob centers. Here, the average density of CO staining at different distances from labeled cells was calculated. Thus, if cells are concentrated in CO blobs, CO staining density will be higher than average near labeled cells, leading to a dark spot of the same size, shape and orientation as an average CO blob at the origin of the plot. Thus, correlations in CO staining and labeling density due to alignment with the overlying blobs/interblobs can be distinguished from any chance correlations due to periodicities different from that of the overlying CO blobs. A more detailed discussion of the analysis is given elsewhere (Boyd and Casagrande, 1999). The digitally captured CO images and the computer-generated images were printed on a Tektronix Phaser 1040 dye sublimation printer.

Results

Radial Organization

Figure 1 shows a coronal section (AP= -3 mm) through the center of a WGA–HRP injection site placed in 21a. Note that the aggregate injection site is located on the lateral edge of the suprasylvian gyrus and confined to grey matter, with minimal involvement of white matter. Although the main purpose of the low-power micrograph in Figure 1 is to demonstrate the extent of a typical aggregate injection site, retrograde labeling in area 17 (open arrow), area 18 (solid arrow) and in area 19 (arrowheads) is also evident. Figure 2 shows, at higher power, labeling in area 17 and 18 from the same experiment shown in Figure 1. Figure 2B illustrates the patchy nature of efferent neurons projecting to area 21a. The border between area 17 and area 18 (large arrowhead), was identified by cytoarchitectonics using a Nissl counterstain. The patches of label in area 18 are wider and less distinct than those seen in area 17. Earlier studies described the patchy nature of the 21a projecting neurons in area 17 after small injections in 21a (Symonds and Rosenquist, 1984b; Sherk, 1986). However, this is the first study that has shown that this projection is patchy even after multiple injections were used to cover the full expanse of 21a, lending support to the hypothesis that the connections between area 17 and 21a are organized in a truly discontinuous fashion.

The population of efferent neurons projecting to 21a formed a diverse group of small, medium and large pyramidal cells located primarily in layer 3. In addition to the labeling in the supragranular layers, we noticed a sparse number of labeled cells in layers 5a and 6 after 21a injections. Cells in the infragranular layers were located within the same cortical column that contained the supragranular labeled cells (Fig. 2, small arrowheads). Consistent with other studies, the infragranular cells represent a minor part of this projection (Dreher et al., 1996a).

Figure 2B shows a single patch of labeling at higher power. The 21a-projecting neurons include a subset of the large border pyramids at the layer 3/4 border (O’Leary, 1941), as well as many smaller cells higher in layer 3. A zone of decreased density of 21a-projecting cells immediately above the level of the border pyramids suggests a trend towards a bimodal laminar distribution within layer 3. Note that many of the cells that are present in this zone of decreased density of 21a-projecting cells are only lightly labeled compared the deeper and more superficial cells. This lighter labeling suggests that they may have only minor projections to 21a, perhaps as collaterals of a more robust projection. The sublaminar organization of 21a-projecting cells within layer 3 was compared to that of LS-projecting cells using fluorescent double-retrograde labeling techniques and confocal microscopy. Figure 3A,B show project images of cells in the same cortical column of a 250 µm thick section projecting to either 21a or LS respectively. The sublaminar organization is best seen with the help of the two reference lines in Figure 3, which divide layer 3 into three equal sublayers. Histograms of the percentages of labeled cells as a function of cortical depth were generated for 21a- (Fig. 3A, left) and LS- (Fig. 3B, right) projecting neurons shown in the double-labeled project image. The percentages of 21a-projecting cells in the upper, middle and lower portions of layer 3 were 37%, 27% and 36% respectively. The percentage of cells in the middle subdivision would be lower if the lightly labeled cells were excluded from the analysis. The LS-projecting cells were more abundant in the middle (44%) and lower (40%) portions, with fewer cells in the upper (11%) portion of layer 3. Thus, the LS-projecting neurons have a different, although overlapping, distribution. While both 21a- and LS-projecting neurons are numerous in the bottom third of layer 3, 21a-projecting neurons are sparse in the middle third of layer 3 while LS-projecting cells are sparse in the upper third of layer 3. Although the lower part of layer 3 gives rise to projections to both 21a and LS, it is important to note that none of the cells in Figure 3 project to both areas. In the three double-labeling experiments only three double-labeled cells were found that projected to both 21a and LS.

Tangential Organization

The efferent cells projecting to 21a were also studied in the tangential plane. Figure 4A illustrates a dark-field photomicrograph of a tangential section taken through layer 2/3 after an aggregate injection of WGA–HRP into 21a. Note the regular array of patchy labeling seen over a 5 × 5 mm area of visual cortex. Also evident in Figure 4A are three or four larger, irregular patches of label in the upper left corner, that most likely represent 21a-projecting neurons in area 18. The assignment of this labeling to area 18 is consistent with the estimation of the border between areas 17 and 18 based on characteristic CO-staining patterns from alternate sections and by gyral patterns evident in the flattened cortex. The larger size of these patches, seen here and in the coronal plane in Figure 2B, appears to be consistent with the trend towards a generally larger-scale modularity in area 18 (Boyd and Matsubara, 1999). Examination of the injection site in this case (not shown) revealed that the multiple injections had
fused into one continuous zone of uniformly dense reaction product. Thus, the patchiness of the labeling in areas 17 and 18 was not due to differences in the uptake of tracer due to a non-uniform injection site.

The labeled cells shown in Figure 4 were charted over alternate serial sections through the superficial layers of area 17 and displayed as a composite cell chart (Fig. 4B) and as a cell density graph for several sections (Fig. 4C). The density of cells...
ranged between 0 and 3600 cells/mm². The overall density can be estimated to be approximately double the numbers quoted, as only alternate serial sections were processed for WGA-HRP. A transect line (D in Fig. 4C) taken through a sample area of adjacent patches revealed that the center-to-center patch spacing was between 0.6 and 0.8 mm.

The profile plot of the transect line in Figure 4D also shows the ‘tightness’ of the clustering, i.e. the difference in labeling density inside the clusters compared to the labeling density outside the clusters. A comparison of this data with similar data from our recent study of projections to LS (see Figs 4 and 5 of Boyd and Matsubara, 1999) suggested that the cells projecting to area 21a might be more tightly clustered than the cells projecting to LS. We developed a simple ‘clustering index’ to compare the density of labeled cells inside and outside of the cell clusters. Our clustering index was similar to Shipp and Grant’s (Shipp and Grant, 1991) ‘index of patchiness’ except that it was done on tangential instead of coronal sections and was normalized from zero to one.

Figure 5 shows four examples of the clustering analysis, two each for 21a- and LS-projecting cells. As described in Materials and Methods, a user-independent method was used to determine the borders of the cell clusters. The black, white and gray areas of each panel of the figure represent the darkest, middle and lightest thirds of an image resulting from Gaussian blurring and low-pass filtering the charted cells (shown as red dots). The black area was then used to define the positions and extent of the clusters, and the white area that of the inter clusters. The density of cells in the clusters (a) and inter clusters (b) was then used to calculate the clustering index: \((a + b)/(a - b)\). The indices of clustering for the examples of 21a-projecting cells in this figure (0.78 for Fig. 5A and 0.67 for Fig. 5C) were greater than the indices of clustering for the examples of LS-projecting cells (0.37 for Fig. 5B) and (0.44 for Fig. 5D). Indices of cluster-
ing were calculated for two other examples of 21a-projecting cells (0.69, 0.72) and two additional examples of LS-projecting cells (0.48, 0.41) (not shown). Indices of clustering were always greater for 21a-projecting cells than for LS-projecting cells, showing that 21a-projecting cells are more tightly clustered than cells projecting to LS.

**Relationship to Cytochrome Oxidase**

Our previous study (Boyd and Matsubara, 1999) showed that the patches of LS-projecting cells are located in CO blob columns in area 17. The overlap of columns of cells projecting to 21a and LS (Fig. 3) suggested that 21a-projecting cells might also be localized to CO blobs. By reacting alternate serial sections for CO histochemistry, or for retrograde tracers, we were able to address directly the coalignment between patches of 21a efferent neurons and the CO blobs. Figure 6 illustrates an experiment where CTX-gold was injected into 21a and the tissue was sectioned in the coronal plane. The first panel (Fig. 6A) shows a photograph of cell labeling in area 17 along the lateral gyrus in a single section. When compared with the CO staining in an adjacent section (Fig. 6B), it can be seen that there is a general correspondence between the density of retrogradely labeled cells and the density of the CO histochemical reaction. This correspondence between patches of 21a-projecting cells and CO blobs can be seen more clearly in Figure 6C, which shows the labeling pattern superimposed on the CO staining.

The correspondence between CO blobs and patches of 21a efferent neurons in area 17 was even more strikingly evident in the tangential plane. Figure 7A shows a bright-field photomicrograph of a section from the same WGA–HRP experiment illustrated in Figure 4, showing the typically patchy labeling pattern of 21a-projecting cells. Figure 7B shows a slightly deeper section from the same experiment, stained for CO. Note the areas of darker and lighter CO staining, i.e. blobs and interblobs. The two sections were aligned using radially penetrating blood vessels (some of which are marked by arrows). Using the arrows for alignment, the positions of cell clusters and CO staining can be compared between Figure 7A and Figure 7B. Note that most of the patches of labeled cells align with CO-dense blob in the CO-stained section.

As described in Materials and Methods, a three-way chi-squared analysis was performed on the data in Figure 7 by dividing the cortex into three approximately equal sized compartments: blob, blob borders and interblobs. In Figure 7C, the blobs are shown outlined and the retrogradely labeled cells (black dots) are superimposed. Only the outlines of blobs are

![Figure 5](image_url)

**Figure 5.** While both the 21a- and LS-projecting cells were patchy, the 21a-projecting cells (A,C) in area 17 demonstrated ‘tighter’ clustering than the LS-projecting cells (B,D) in area 17. The cell charts were subjected to a Gaussian blur, low-pass filtered and thresholded into thirds to define cell clusters (the darkest third), interclusters (the lightest third) and cluster borders (the intermediate third). The density of cells in the clusters (a) and the interclusters (b) was then obtained and a clustering index calculated by the following algorithm: \( \frac{a + b}{a - b} \). Using this method, a population in which the patches were ‘tightly’ clustered would have a value closer to 1, while those populations in which the cells were less tightly clustered would have values closer to 0. (A,C) The reconstructed pattern of labeled cells (red dots) superimposed on the cell clusters (black), cell interclusters (white) and cluster borders (gray) for two separate cases for the 21a efferent population in area 17. (B,D) illustrate data from two separate cases for the LS efferent population. The indices of clustering for the 21a cases shown here were 0.78 (A) and 0.67 (D), while the indices for the LS cases were 0.37 (B) and 0.44 (D).
shown for clarity, so the remaining cortical area includes both interblobs and blob borders. For this experiment, the density of cells in the blobs was 549 cells/mm$^2$, the density of cells in the interblobs was 258 cells/mm$^2$ and the density of cells in the blob borders was 394 cells/mm$^2$. The density of 21a-projecting cells in the blobs was thus more than twice as great as the density of 21a-projecting cells in the interblobs. The $P$-value of the chi-squared analysis for this data was <0.0001, showing that more labeled cells were found in the blob columns than would be expected by chance.

The relationship between the 21a-projecting cells and the CO architecture in this experiment was also studied using the two-dimensional spatial cross-correlation techniques described in Materials and Methods. Figure 7D shows the correlation between CO staining and 21a-projecting cells. The reference position, (0,0), is in the center of the figure, and the darkness of the image at different x,y-distances from the reference position corresponds to the CO-staining density at that distance averaged for all of the 21a-projecting cells. The dark area near the center of the image indicates that 21a-projecting cells are found preferentially in areas of darker CO staining. As the size and spacing of the dark area in the center of the image, and the dark areas along the edges of the image (caused by the regular periodicity of the CO blobs) are the same as the size and spacing of the CO blobs, it can be concluded that the pattern of 21a-projecting cells shows a relationship to the CO architecture. The fact that the dark area is not centered exactly on the reference position is probably caused by imprecision in aligning the 21a-projecting cells with the CO image, which was taken from a section deeper in the cortex than the sections containing most of the labeled cells.

The two-dimensional cross-correlation in Figure 7D was collapsed radially onto a single dimension and divided into 0.1 mm bins, giving the plot shown in Figure 7E, where the average CO-staining value is plotted as a function of distance from the reference position. The CO-staining value is lowest (darker staining) closest to the reference position, and gradually increases, leveling off at ~0.3 mm away from the reference position. Although the original CO image was an 8-bit image, i.e. it had values ranging from 0 to 255, the range of values in the correlation result is much smaller due to the averaging involved.

A second experiment in which 21a was injected with CTX-gold and the cortex was sectioned tangentially was analyzed in the same fashion (Fig. 8). Figure 8A, B shows retrograde labeling and CO staining respectively, with the positions of patches of labeled cells marked by asterisks. Again, the correspondence between the patches of labeled cells and the CO blobs is evident. As for the previous experiment, the cortex was thresholded into blobs, interblobs and blob borders, and the extents of the blobs are indicated in Figure 8C. For this experiment, the density of cells in the blobs was 190 cells/mm$^2$, the density of cells in the interblobs was 46 cells/mm$^2$ and the density of cells in the blob borders was 86 cells/mm$^2$. In this experiment, the differential in labeling density between blobs and interblobs was more pronounced than in the previous one, with more than four times as great a density of 21a-projecting cells in the blob borders. Again, these results were significantly different ($P < 0.001$) from chance using chi-squared analysis.

The two- and one-dimensional versions of the spatial cross-correlation plots for this experiment are shown in Figure 8D, E respectively. There is a dark area in the center of the two-dimensional plot, showing that 21a-projecting cells in this experiment tended to be in areas of high CO density, and there are secondary dark areas at the edges of the plot, representing adjacent CO blobs. Again, these results show that 21a-projecting cells tend to be found in CO-blob columns.

**Discussion**

It was Semir Zeki (Zeki, 1975, 1993) who first advanced the...
idea that the circuitry within area 17 might be responsible for generating several different classes of functionally distinct outputs and channeling these outputs into the appropriate extrastriate areas. In this paper, we have demonstrated both differences and similarities in the anatomical organization of two output pathways from area 17 to the functionally distinct areas 21a and LS. Yet, it is not obvious how the differences/similarities in the anatomical organization of these pathways relates to any functional differences/similarities in the projection to these two areas.

**Columnar Comparison**

Both the projections to LS and to area 21a originate from discontinuous patches in area 17 that align with CO blob columns. In this study, as well as earlier studies on connections to LS (Shipp and Grant, 1991; Ferrer et al., 1992; Boyd and Matsubara, 1999), multiple injections of retrograde tracers were used to produce large aggregate injection sites in order to include all possible types of columns in 21a or LS, yet even the largest injections did not lead to continuous labeling in area 17. However, the patchy projections to area 21a and LS differ in how tightly they are clustered into the patches, with 21a-projecting cells being more tightly clustered than LS-projecting cells. In the context of results suggesting that CO blobs and interblobs have different functional properties based on their different LGN inputs (Hubener et al., 1977; Shoham et al., 1997; Boyd and Matsubara, 1996), it would be tempting to suggest that the cells projecting to LS might have access to a similar but slightly broader range of inputs compared to those projecting to 21a due to the greater proportion of LS-projecting neurons found between the cell clusters aligned with the CO blobs. If, for whatever reason, the blob/interblob distinction was stronger in different sublayers of layer 3, then the different sublaminar location of 21a- and LS-projecting cells might also be responsible for the difference in tightness of clustering between these two projection populations. Due to the difficulty of determining sub-

**Figure 7.** Analysis of 21a-projecting cells and CO staining in the tangential plane. (A) A photomicrograph of 21a-projecting cells in area 17. (B) An image from a CO-stained section aligned with the data from (A) using blood vessels (arrows) as landmarks. Note that most of the patches of cells aligned with a CO blob. Scale bar = 1.0 mm. (C) Enlargement of the same CO image as in (B), with the positions of the labeled neurons charted from several sections represented by black dots. The borders of the CO blobs, determined using the procedure described in the text, are indicated by solid lines. These borders were used to calculate a chi-squared statistic (see text) which showed a significant concentration of 21a-projecting cells within the CO blobs in this experiment. Scalebar = 1 mm. (D) Two-dimensional spatial cross-correlation of the data shown in (C). The darkness of the image at each x,y offset is proportional to the density of CO staining at that offset averaged over all the labeled cells. The dark spot near the origin of the plot (marked with cross-hairs) shows that CO staining was darker near labeled cells. (E) One-dimensional spatial correlation between distance from labeled cells and CO-staining density.
laminar location in tangential sections, we have not addressed this question, but it would be of interest to calculate separately the tightness of clustering for the upper tier of 21a-projecting cells versus the lower tier.

**Sublaminar Comparison**

How might the differences in laminar distributions of LS— and 21a-projecting cells in the CO blobs contribute to functional differences in the output to these two areas? Our earlier studies demonstrated that the CO blob columns in cat visual cortex mark the termination sites of geniculocortical afferents from the C-laminae of the lateral geniculate nucleus (LGN). Y-cells from layer C terminate in layer 4A of the blobs, and W-cells from the parvcellular C-layers terminate in layer 3 (Boyd and Matsubara, 1996). The tier of LS- and 21a-projecting cells in the bottom third of layer 3 are thus both placed to receive Y-cell input on their basal dendrites. Yet selectively blocking the Y-channel via a unilateral optic nerve pressure block shows smaller changes in the responsivity of 21a neurons compared to neurons in LS (Dreher et al., 1993, 1996b; Wang et al., 1997). Presence of Y-cell input to 21a is also argued for by the numerous inputs to this area from 18 (Fig. 2), an area that is known to be dominated by Y-cell input from the LGN (Stone, 1983). It is possible that convergence of inputs from other non-Y sources onto cells in 21a may have larger effects, partially masking the presence of the Y-cell input.

The second population of 21a-projecting cells in area 17 appear to be located superficial to the sites of geniculocortical termination (Boyd and Matsubara, 1996). These cells may differ from the LS population by not receiving direct geniculate inputs. If this is true, it would suggest that a subclass of information going to 21a might traverse a further stage of processing in area 17, compared to that going to LS. Perhaps the intrinsic interlaminar connections that provide the input to the upper tier of 21a-projecting neurons endow these cells with a different set of functional properties than the lower tier of 21a-projecting cells.

Unfortunately, we know of no study suggesting a correlation of functional parameters such as direction/orientation selectivity or spatial/temporal tuning with cortical depth within the supragranular layers. Nor have there been any studies looking at...
differences in the interlaminar connections of the sublayers of layer 5 in cat area 17, as has been done in several primate species (Lachica et al., 1992, 1993; Yoshioka et al., 1994). Ultimately, studies combining electrophysiological examination with identification of different classes of projection neurons (Movshon and Newsome, 1996) will be required to examine functional differences in the anatomically distinct output pathways like the ones shown in this study.

Notes
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