Brain Research, 560 (1991) 207–215

ADONIS 000689939116999G

BRES 16999

Intrinsic connections in cat visual cortex: a combined anterograde and retrograde tracing study

Jamie Boyd[1,3] and Joanne Matsubara[1–3]

Departments of [1]Ophthalmology and [2]Anatomy and [3]The Graduate Program in Neuroscience, University of British Columbia, Vancouver, B.C. (Canada)

(Accepted 30 April 1991)

Key words: Patchy connection; Lamina; Column; Reciprocity; Visual cortex

Area 18 of cat visual cortex was examined for intrinsic axons following small, columnar injections of an anterograde tracer, *Phaseolus vulgaris* leucoagglutinin (PHA-L). Locally projecting axons radiated from the injection site and branched to form 10–15 discrete, approximately circular patches 500–750 μm in diameter consisting of many bouton-studded terminal arborizations. Labeled fibers and boutons ramified densely in layers I, II/II, V, and VI, and were noticeably less dense in layer IV. Afferent and efferent pathways originating from the same cortical columns were studied by injecting a mixture of PHA-L and wheat germ agglutinin conjugated to horseradish peroxidase (WGA–HRP). Between 10 and 15 patches of cells retrogradely labeled by WGA–HRP surrounded each injection site. Within a patch, labeled cells were found in all layers and included both pyramidal and non-pyramidal cells. The distribution of PHA-L labeling was similar to that obtained when PHA-L was injected alone. Most often, the labeled patches resulting from injections of such mixtures contained both anterograde and retrograde labeling. However, patches consisting of retrograde labeling alone and of anterograde labeling alone were also observed, indicating that the local connections linking neighboring cortical columns were not always reciprocal.

INTRODUCTION

In many species, an injection of retrograde tracers into a small region of cortex produces both patches of labeled cells at corresponding sites in other cortical areas, and patchy patterns of locally labeled cells extending several millimeters from the injection site[22]. Thus, intercolumnar interactions within the visual cortex are mediated both by connections between different cortical areas, and by local connections that are intrinsic to a single area, as has been demonstrated in the tree shrew[35], primates[23,33,34] and cats[14,24,27].

We surmised that the similarities between local and corticocortical connections would extend beyond the common patchy distribution pattern. Local connections in cat visual cortex were examined for evidence of two further features of corticocortical connections: a projection-specific, laminar distribution of efferent axons and afferent cell bodies, and pathway reciprocity.

In order to examine the reciprocity of local connections, it is necessary to study the anterograde and retrograde pathways arising from a single injection site. In theory, this examination should be possible using a bidirectional tracer such as wheat germ agglutinin conjugated to horseradish peroxidase (WGA–HRP). In practice, this technique fails because WGA–HRP may label the axonal collaterals of retrogradely labeled cells as intensely as the terminal fields of cells in the injection site[8,16,42], thus confounding the results. In order to circumvent this problem, WGA–HRP has been used in combination with an anterograde tracer, such as tritiated amino acids, to study areal reciprocity[4,17,30,32,44,50,51,54].

In our experiments, we used a modified version of this method and employed *Phaseolus vulgaris* leucoagglutinin (PHA-L)[12] as the anterograde tracer in place of the tritiated amino acids. For the purposes of this study, PHA-L offered two main advantages over tritiated amino acids: (1) it fills axons completely, including the axonal arborizations and boutons, making possible the distinction between terminal fields and fibers-of-passage; and (2) it allows for the visualization of WGA–HRP in the same section of tissue. Because of these advantageous properties, PHA-L was used to examine the termination pattern of local projections in the coronal and tangential planes.

This study shows that local projections terminate in all cortical layers, though much less densely in layer IV, while the locally projecting cells were found in all cortical layers. Because local connections originate in both infragranular and supragranular layers, and terminate in

Correspondence: J. Boyd, Department of Ophthalmology, Rm 330, 2550 Willow St., Vancouver, B.C. V5Z 3N9, Canada.

both upper and lower layers as well, they resemble the so-called 'lateral' connections made between cortical areas at the same level of the processing hierarchy[28,56]. Finally, this study suggests that intrinsic connectivity is made up of both reciprocal and non-reciprocal connections. A preliminary report of some of these results has appeared[2].

MATERIALS AND METHODS

Adult cats, both males and females, weighing from 2.5 to 4 kg were used in these experiments. Two cats were given cortical injections of PHA-L and their cortices were sectioned in the coronal plane in order to examine the laminar distribution of local efferents. Six cats were injected with a mixture of PHA-L and varying concentrations of WGA–HRP and their cortices were sectioned tangentially in order to examine the reciprocity of local connections. In 4 of these cases, alternate sections were used to visualize the two tracers and in the remaining two cases, both tracers were visualized in each section using a double labeling protocol.

The animals were anesthetized with i.v. alphaxalone/alphadolone (Saffan; Glaxovet) and were then given atropine i.v. and dexamethasone i.m. to reduce respiratory secretions and brain edema, respectively. Anesthetized animals were placed in a stereotaxic frame and a craniotomy was performed at stereotaxic coordinates +1 to +4 mm M-L and −1 to +6 mm A-P, which correspond to the lower visual field of area 18[47].

PHA-L (Vector Labs) was reconstituted as a 2.5% solution according to the manufacturer's instructions and then used either unadulterated or mixed with enough crystalline WGA–HRP (Sigma Chemicals; type VI) to give a 1–8% WGA–HRP concentration. The tracer solution was drawn into a glass pipette, the tip of which measured between 10 and 20 μm in inner diameter and the pipette was then lowered to a depth of 1 mm into the cortex through a small slit made in the dura. The positive terminal of a constant current source (Transkinetics) was connected to a silver wire in the pipette and the negative lead was lightly clipped to the animal's tongue. Positive-pulsed current (5 μA; 7 s on, 7 s off) was applied for 25–30 min. to iontophorese the tracers. Only one injection was made into each animal to avoid confusing interhemispheric labeling with the pattern of local labeling.

After allowing 4–5 days for transport, the animal was euthanized with an overdose of pentobarbital (euthanyl; M.T.C. Pharmaceuticals) and perfused transcardially with the following solutions: 1.5 liters 0.5% sodium nitrite in 0.1 M phosphate buffer, pH 7.2 (PB); 2.0 liters 4% paraformaldehyde and 0.1% glutaraldehyde in PB; 10% sucrose in PB. Blocks of tissue containing the visual cortex were dissected out and mounted on the stage of a freezing microtome. Sections were cut at 50 μm thickness in the coronal or horizontal plane and then collected in PB in individual wells.

Transported PHA-L was visualized using an avidin–biotin–peroxidase method (Vector Labs). Sections were incubated in goat anti-PHA-E+L at a dilution of 1:500 or 1:1000 for 14–16 h, rinsed 3 times in PB, and then incubated for 1 h in biotinylated anti-goat IgG. Both antibody solutions contained 1% Triton-X-100 to enhance penetration. After further PB rinses, the sections were incubated in peroxidase labeled avidin–biotin for 1 h and then rinsed in PB. The peroxidase was then visualized with a diaminobenzidine (DAB)–glucose oxidase reaction[18] without cobalt enhancement.

For each of the experiments in which a mixture of tracers was injected, one of two procedures was used. Either the sections were divided into two series, with one series being immunoreacted for PHA-L and the other being used for visualization of the transported WGA–HRP using the tetramethyl benzidine (TMB) method[29], or else all of the sections were reacted sequentially to reveal both tracers. This was accomplished by stabilizing the TMB reaction product with ammonium heptamolybdate followed by cobalt acetate and DAB[15] prior to incubating the sections in the primary antibody against PHA-E+L. This stabilization inactivated any remaining peroxidase activity of the WGA–HRP, and no cross-reaction was ever observed between the two peroxidase reactions. The stabilized TMB reaction product consisted of black granules and was easily distinguished from the diffuse brown DAB reaction product. Sections were mounted onto gelatinized glass slides, air dried, dehydrated through a graded series of alcohols, and then cleared in xylene and coverslipped with DPX.

Using a drawing tube attached to a Nikon Optiphot microscope, detailed charts were made of serial coronal or horizontal sections, the injection site, zones of labeling, and the pattern of blood vessels. For examination of the laminar distribution of local connections, individual axonal swellings (boutons) were drawn as separate points. For the experiments examining the reciprocity of local connections, the analysis was done at lower resolution and individual boutons were not counted. Instead, densely labeled patches of terminal fields were outlined on the charts. Each individual retrogradely labeled cell, however, was drawn as a separate data point. When charting PHA-L or WGA–HRP label in alternate serial sections, the magnification of the drawing tube was adjusted, through comparison of blood vessel patterns in adjacent sections, to account for the slightly greater shrinkage of the TMB reacted sections. These charts were then digitized and, with the injection site and blood vessels serving as landmarks, aligned using custom designed software run on a Compaq 386 computer to produce the composite charts shown in the figures.

RESULTS

Under the conditions of this study, PHA-L iontophoresis resulted in injection sites of approximately 1 mm in diameter. When brains were cut in the coronal plane, the dense core of the injection site which contained darkly labeled cells and probably gave rise to most of the anterograde labeling[12], was found to extend through all cortical laminae.

Patchy termination of locally projecting axons

In all cases, injections of PHA-L into cat area 18 resulted in a pattern of anterograde labeling comprising discrete patches of dense labeling and a matrix of unlabeled or lightly labeled areas. The PHA-L labeling was almost entirely anterograde, but occasional retrogradely labeled cells were seen in addition to anterograde labeling. Fig. 1A shows 4 patches from a case sectioned in the tangential plane. PHA-L-labeled patches were roughly circular, approximately 500–750 μm in diameter, and were separated from the injection site and each other by approximately the same distance. Between 10 and 15 patches resulted from a typical PHA-L injection. It was noted that patches close to the injection site were usually more densely labeled than more distant patches.

Laminar distribution of locally projecting axons

The laminar distribution of the labeled axons and terminals was examined in sections of tissue cut in the coronal plane. Fig. 1B is a photomicrograph of part of a coronal section containing PHA-L labeling. Patches of

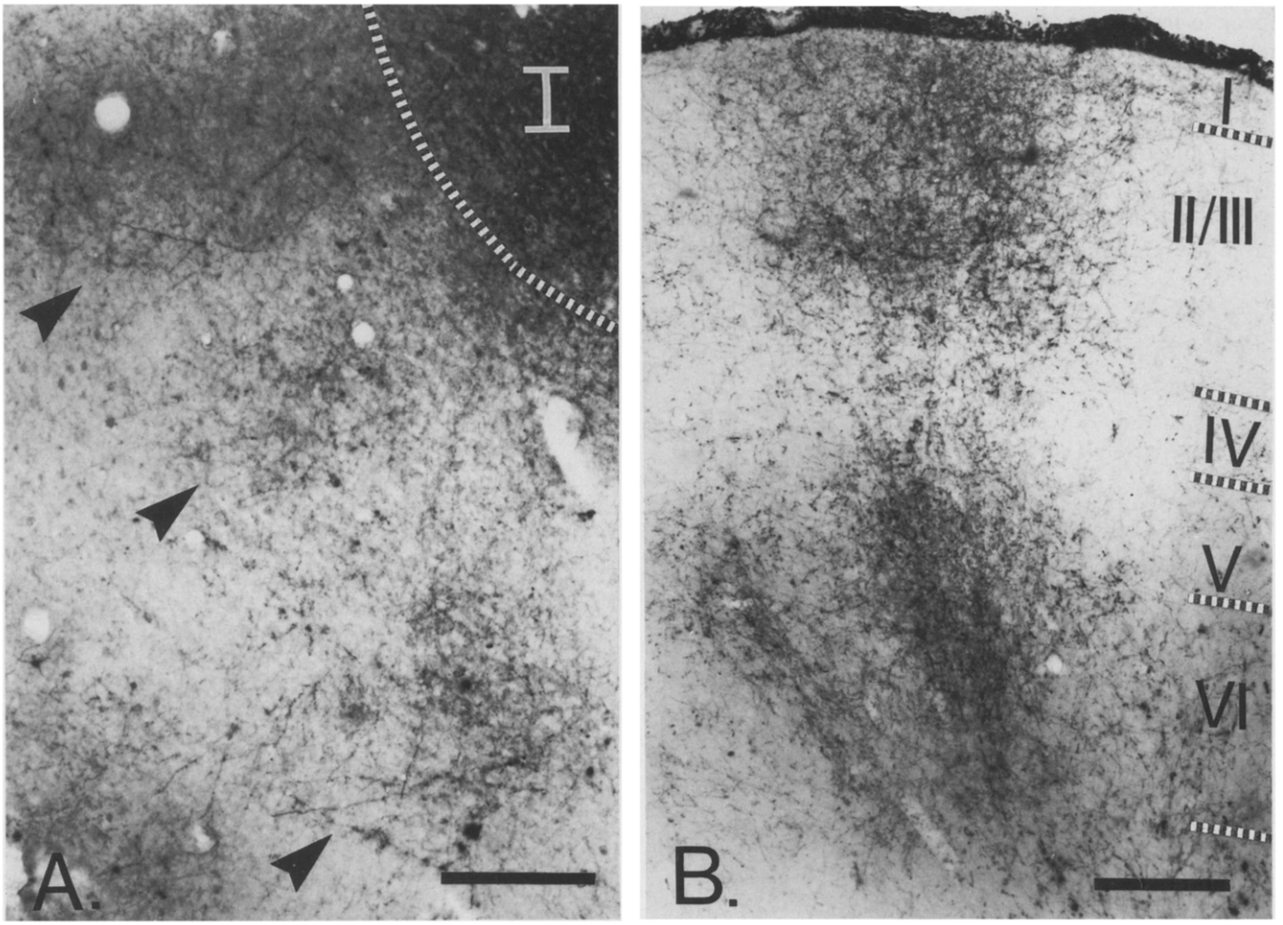

Fig. 1. Patchy intrinsic connections in area 18 of the cat labeled with the anterograde tracer PHA-L. A: photomicrograph of a tangential section through layers II/III. Fibers radiating from the injection site, 'I', outlined with a dotted line, ramify to form discrete patches of dense arborization, 3 of which are marked by arrowheads. B: photomicrograph of one patch of PHA-L labeling in a coronal section. Labeled axons are found in all layers but are much less dense in layer IV than in the other layers. Scale bars in A and B = 250 μm.

dense labeling extended from pia to white matter in a columnar fashion. Within the patches, density of labeled axons and the width of the labeled zone was much less in layer IV than in the other layers. Also, the width of the labeled zone in the infragranular layers was wider than in the supragranular layers.

The PHA-L immunoreaction product filled the labeled axons so well that they resembled intracellularly injected axons. Fig. 2A shows a single labeled axon, within a patch of labeling, branching several times to form a terminal arbor. The width (400 μm) of that part of the arbor contained within one 50 μm thick section is nearly as great as the widths of entire patches, which suggests that labeled axons must be in near-perfect columnar register within a single patch.

Many swellings or boutons were readily apparent along the lengths of the PHA-L labeled fibers and these were probably synaptic terminals[55]. Fig. 2B shows the positions of individual boutons within a single patch relative to laminar boundaries in 4 serial coronal sections. Labeled boutons were least dense in layer IV and were more dense in layer II/III, especially the lower part, than in layers V and VI. The lateral extent of labeled boutons was narrowest in layer IV and was wider in infragranular than in supragranular layers.

Reciprocity of local connections

In order to study the reciprocity of local connections in area 18, solutions containing a mixture of PHA-L and WGA–HRP were injected. While keeping the concentration of PHA-L constant, the concentration of WGA–HRP in the mixture was varied in order to determine the effect on both the overall quality and the extent of the labeling pattern. In all cases, the patchy pattern of retrograde WGA–HRP labeling was similar to the pattern previously described[27]. An increase in the concentration of WGA–HRP from 1 to 2% resulted both in increased numbers of cells in each patch of labeling, as well as more retrogradely labeled patches. While an increase in the concentration of WGA–HRP above 2% did not ap-

pear to increase the number of patches, higher concentrations did increase the number of cells in each patch. Furthermore, an increase in concentration from 2 to 8% slightly raised the intensity of staining within the WGA–HRP injection site, without seeming to increase its diameter.

The patches of anterograde PHA-L labeling resulting from the mixed injections were similar to those described for the injections of PHA-L alone. As the concentration of WGA–HRP in the mixture was increased, the amount of PHA-L labeling in each patch decreased, without decreasing the total number of patches. The PHA-L labeling decrease was probably due to higher proportions of the iontophoretic current being carried by ejection of WGA–HRP causing less PHA-L to be ejected, or to competition between the two tracers for uptake and transport sites[46].

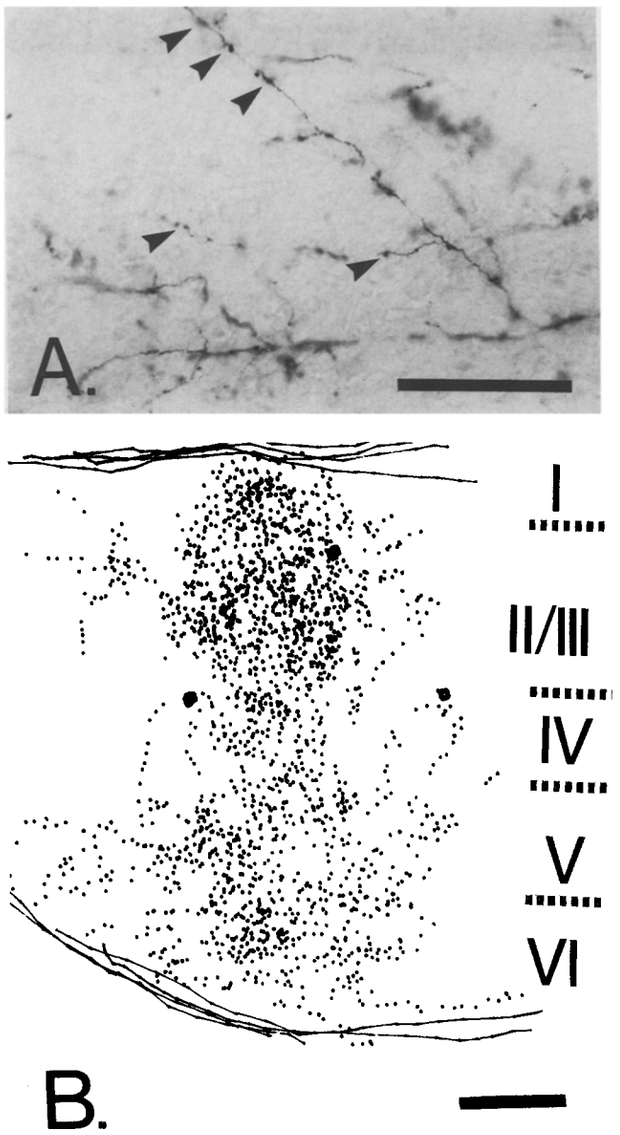

Fig. 2. The appearance and laminar distribution of axonal boutons in the PHA-L labeled tissue. A: photomicrograph of a single PHA-L labeled axon from layer II/III. Micrograph is oriented with pia to the top. The axon branches to form a terminal arbor studded with boutons, some of which are marked with arrowheads. The terminal arbor occupies a space of approximately 400 μm, nearly as wide as the entire patch of labeling in which it was located. Scale bar = 75 μm. B: aligned charts of 4 serial coronal sections through the center of a different, less dense patch of labeling than shown in B. Each dot represents a single bouton. Within a patch, boutons are numerous in all layers, but are least common in layer IV. Scale bar = 100 μm.

Fig. 3A and B are composite charts of labeling for two of the combined injection experiments. As can be seen, the distribution of labeled cells and axons overlapped in many patches. However, there were distinct areas where the retrograde and anterograde labeling did not coincide. Thus, we observed zones containing labeled cells without axon labeling, and other zones with axon labeling but without labeled cells. As shown in Fig. 3, our results regarding reciprocity of local connections were not affected by increasing the concentration of WGA–HRP above 2%. Notwithstanding this lack of complete overlap, retrograde and anterograde connections were seen to extend about the same distance (6–8 mm) across the surface of the cortex, while the numbers of patches (10–15) labeled by the two tracers were seen to be roughly equal.

In some experiments, WGA–HRP-reacted sections were stabilized and subsequently immunoreacted for PHA-L. The visualization of both anterograde and retrograde labeling within the same section ensured that inaccuracies in aligning the two sets of sections did not affect our assessment of reciprocity, and also allowed us to identify PHA-L labeled axons that were contacting WGA–HRP labeled cells.

The photomicrographs shown in Fig. 4 were all taken from the same representative case illustrating the 3 observed patterns of labeling. In this experiment, each section of tissue was reacted for both WGA–HRP and PHA-L. Fig. 4A illustrates WGA–HRP labeled cell bodies and no axonal fibers or boutons, thus indicating an afferent patch. Fig. 4B shows an efferent patch, in which only axonal fibers and boutons were labeled. Finally, Fig. 4C illustrates a reciprocally connected patch, in which both cell bodies and axonal fibers were strongly labeled. Although an electron microscopic investigation would be necessary to confirm the presence of synapses, light microscopic observations did suggest that some locally projecting cells were receiving contacts from locally projecting axons. Fig. 4D and E are high magnification photos taken with Nomarski optics at different focal planes of the cell arrowed in Fig. 4C. Here, labeled axons wrap around the labeled cell and form, in close apposition to it, bouton-like axonal swellings. At other focal planes, several more swellings were visible against the labeled cell. This pattern of termination is typical of

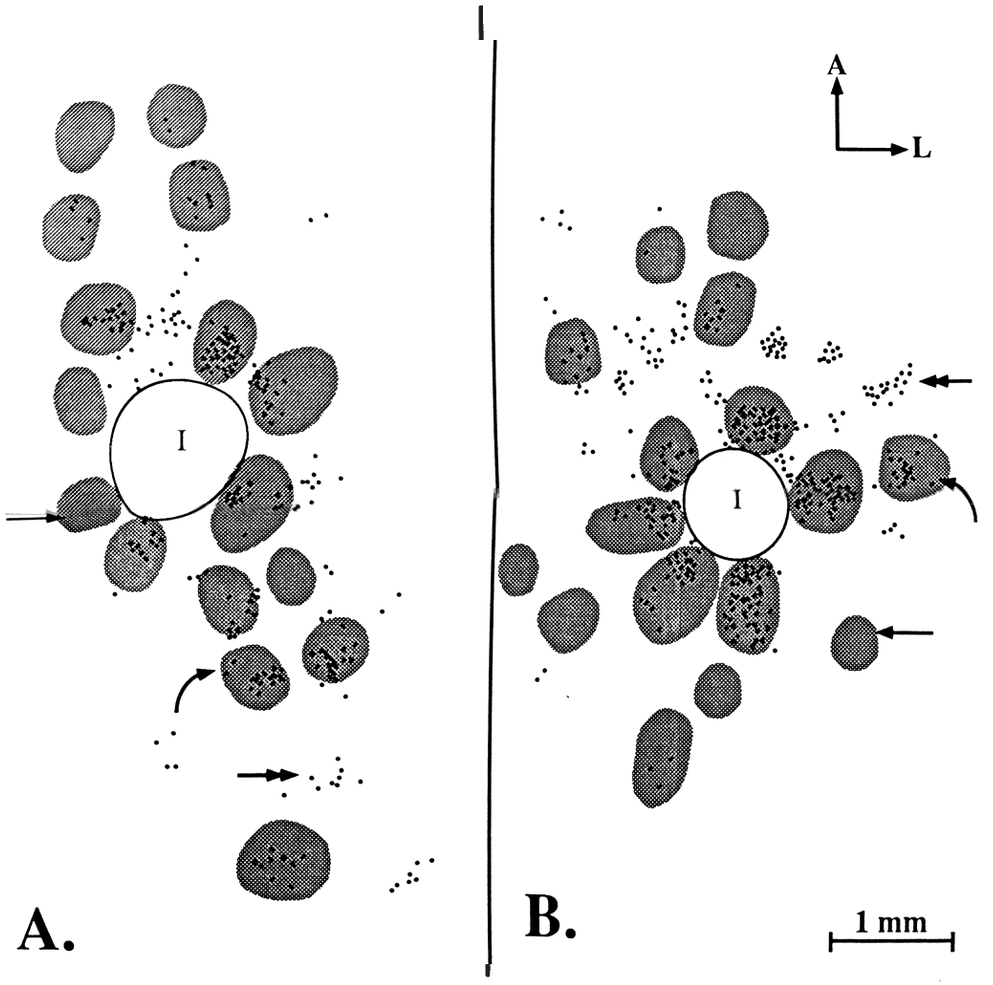

Fig. 3. Aligned charts of retrograde and anterograde labeling in tangential sections from two experiments with injections of PHA-L/WGA–HRP mixtures. The two tracers were visualized in alternate sections and the resulting labeling patterns were digitized and aligned by computer. Each dot represents a retrogradely labeled cell and the shaded areas represent patches of dense anterogradely labeled terminal fields. A: in this experiment, a mixture of 2% WGA–HRP and 2.5% PHA-L was injected. Many of the patches of retrograde labeling are matched by corresponding patches of anterograde labeling (single arrow). However, some areas containing retrograde labeling do not contain anterograde labeling (double arrow) and there are some areas containing anterograde labeling alone (curved arrow). B: in this experiment a mixture of 8% WGA–HRP and 2.5% PHA-L was injected. The higher concentration of WGA–HRP in the injected mixture resulted in more labeled cells but the patchy spatial pattern of labeling is similar to that in A. Again, it can be seen that while many labeled areas contain both tracers, there are patches consisting of only retrograde and others of only anterograde labeling. Scale bar = 1 mm and applies to both A and B. A, anterior; L, lateral; I, injection site.

basket cells[9,37], a type of inhibitory cortical neuron with horizontally projecting axons. With reference to the few putative contacts that were found between labeled cells and axons, it should be noted that the WGA–HRP reaction product labeled only the cell body and proximal dendrites. Thus, contacts on distal dendrites and dendritic spines, where most excitatory synapses are made[5] could not be identified in this study.

DISCUSSION

Distribution of locally projecting axons

The observed distribution of locally projecting axons complements the results obtained by intracellular dye injections[13]. Individual collaterals (Fig. 2) form patches of boutons with similar dimensions to those of PHA-L-labeled patches. As each patch of PHA-L labeling probably comprises the axons of many different cells at the injection site, there must therefore be a considerable amount of overlap between axons in a single patch. The amount of precision in this overlap suggests that projections of different cells within the same column are regulated together somehow.

Previous studies have shown that cells that make patchy intercolumnar projections include spiny stellate cells in layer IV projecting to layers II/III; pyramidal cells in layers II/III projecting to layers II/III and V; pyramidal cells and large basket cells in layer V projecting

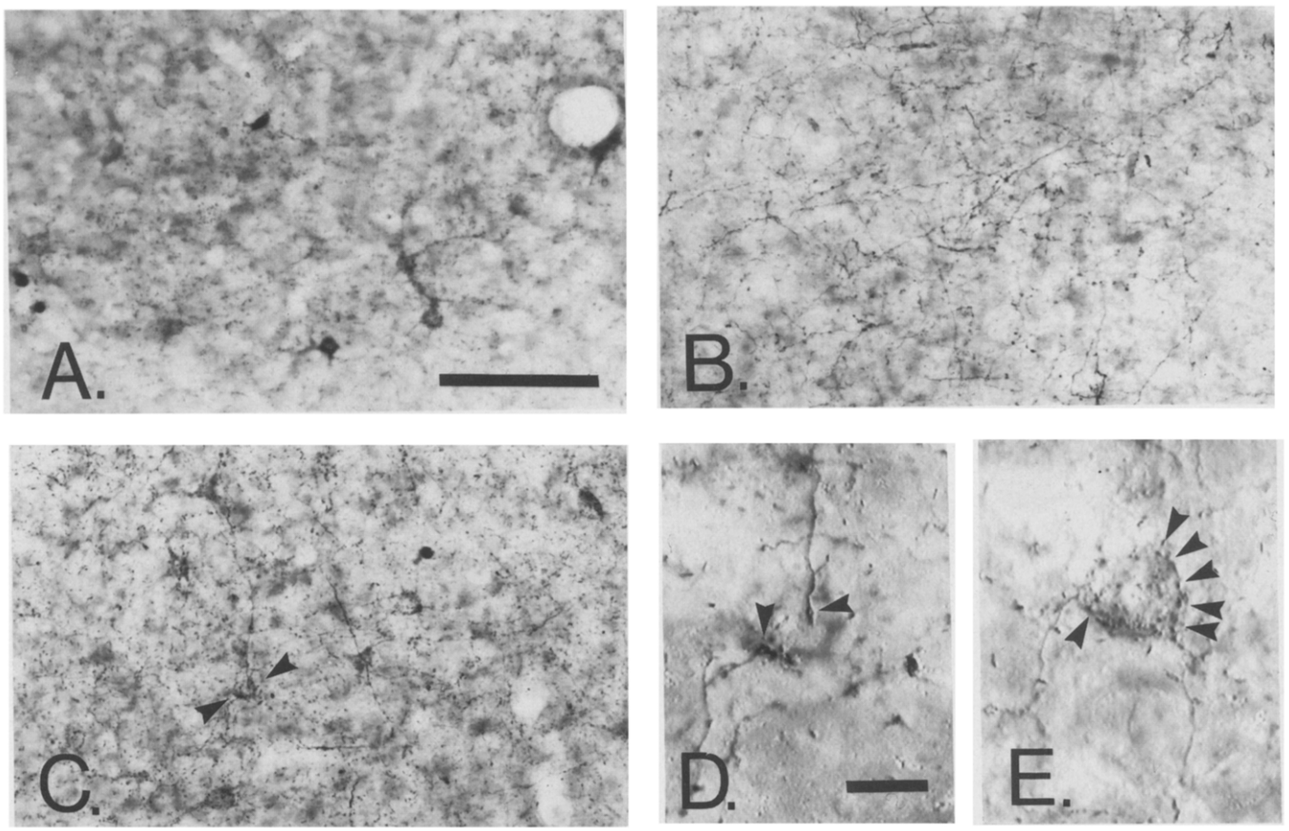

Fig. 4. Photomicrographs of anterograde and retrograde labeling in tangential sections following an injection of 4% WGA–HRP and 2.5% PHA-L. Sections were reacted for HRP, then stabilized and subsequently immunoreacted to visualize PHA-L, allowing both tracers to be localized in the same section. Patches containing only retrogradely labeled cells (A) and patches containing only anterogradely labeled axons (B) were seen, in addition to patches containing both cells and axons (C). The retrogradely labeled cell marked with arrowheads in C is shown at higher magnification using Nomarski optics in D (focal plane at upper surface of cell, so WGA–HRP reaction product in cell is not clearly visible) and E (lower focal plane). Some of the boutons of the anterogradely labeled axons (arrowheads) are in close contact with the cell. Scale bar in A = 50 μm and applies to A–C. Scale bar in D = 5 μm and applies to both D and E.

to layers V and VI; and layer VI pyramidal cells projecting to layer IV[11,20,25,37]. Although there are no previously noted examples of cells making patchy, intrinsic connections to layer I, we did find some terminals in this layer within the labeled patches. Our results also provide some information on the relative strengths of each class of local projections that cannot be gained from single-cell studies, as we have shown that local connections are densest in layer II/III and least dense in layer IV.

The lower density of labeling in layer IV suggests that the feedback projection from layer VI to layer IV is less well developed for the patchy horizontal connections between columns, than for the radial connections within a single column. This finding is reinforced by retrograde labeling studies; local patches of retrogradely labeled cells contain fewer cells in layer VI than in other layers[27].

In our study, each patch contained labeled terminals in all layers. This is interesting in light of the fact that cells in different layers may have different receptive field properties[13], and that the local projections made might thus be expected to serve different functions. One probable correlation between laminar specific receptive field properties and projections was noted; within a single patch the horizontal spread of labeling was most narrow in layer IV and widest in layers V and VI; the same pattern was noted for patches of retrogradely labeled cells[27]. This correlates well with receptive field sizes, which are smallest in layer IV, larger in supragranular layers and larger still in infragranular layers[13].

Reciprocity of local connections

The results obtained from combined injections of WGA–HRP and PHA-L suggested that, while most local connections within cat area 18 were reciprocal, many were not. This aspect of local circuitry has not been described before. As has been noted earlier, one of the pitfalls of using WGA–HRP alone to investigate cortical circuitry is that the filling of collateral terminals within the same column as retrogradely labeled cell bodies may

result in the erroneous conclusion that all areas projecting to the injection site also receive a return projection. Nevertheless, even if local circuitry is not completely reciprocal, the injection of WGA–HRP should reveal some areas of strictly terminal labeling, with no nearby cell bodies. In contrast, it has previously been reported[21] that, following an injection of WGA–HRP in area 18, all patches contained both labeled cells and neuropil labeling with no patches being found to consist only of neuropil labeling. It is difficult to reconcile this observation with the present results. The TMB reaction, the procedure most often used to visualize transported WGA–HRP, shows a terminal field as a fine, granular deposit in the neuropil. It is possible that, in previous investigations of local connections using the TMB method, the granular deposit of lightly labeled terminal fields may have been difficult to identify and was thus missed.

Although the injection of a mixture of two tracers, one anterograde and one retrograde, avoids the problems associated with WGA–HRP anterograde labeling, other problems may be created. To address the issue of reciprocity, it is necessary that effective injection sites (the areas from which tracer is actively taken up and transported) be equal in size for the two tracers. If the effective injection sites of the two tracers in the mixture are mismatched in size, with, for example, a larger anterograde injection site, more patches might be expected to be labeled by the anterograde tracer than from the retrograde tracer and these extra patches might be erroneously categorized as being non-reciprocally connected to the injection site. If some patches contain retrograde labeling from the smaller injection but not anterograde labeling from the larger one, however, then these patches must represent true non-reciprocal connections. As areas containing strictly anterograde and areas containing strictly retrograde labeling were seen in all cases in the present study, it is unlikely that differences in injection site sizes alone could account for the results. Because of the problems inherent with proving negative results, however, it is easier to unambiguously show that two columns are connected reciprocally than to show that they are not; reasons can always be postulated as to why one of the tracers did not work fully. Other possible factors such as differing sensitivities of WGA–HRP and PHA-L uptake, transport and visualization procedures might alter the results obtained. However, the fact that varying concentrations of WGA–HRP did not change the finding of non-reciprocal projections again suggests that technical limitations were not responsible for the results.

Comparison with corticocortical connections

It has been established that relatively few corticocortical pathways are 'one way streets' but, rather, most are matched by a reciprocal projection[1,3,10,19,38,39,43,45,48,49,52,53]. In one hodological study encompassing 17 visual cortical areas, all connections were reciprocal[40].

One of a pair of matching projections can be classified as either 'feedforward' or 'feedback', depending on the position of the afferent and efferent regions in the presumed visual processing hierarchy. Feedforward and feedback connections typically have different specific laminar distributions of efferent terminals and afferent cell bodies[28,56]. Feedforward pathways originate in supragranular layers and terminate mainly in layer IV, while feedback pathways originate both in infragranular and supragranular layers and terminate mainly in layers I and VI. Although first discovered in primates, this distinction probably holds for cats and rodents as well[6,41]. Results from other experiments in our laboratory indicate that projections from area 18 to area 17 terminate mainly in layers I and VI, while those from area 18 to area 19 terminate mainly in layer IV and the lower part of layer II/III.

For such pathways as homotypic callosal projections, the feedforward/feedback distinction is difficult to make because the interconnected areas are, by definition, at the same level within the processing hierarchy. These special types of reciprocal connections have been termed 'lateral' and are marked by a symmetric organization in which labeled cells and terminals are found in both infragranular and supragranular layers[56].

Because local connections are also made between columns at the same level of the processing hierarchy, we might therefore expect them to exhibit some of the features of lateral corticocortical connections, and indeed they do. Previous work has shown that locally projecting cells in area 18 are present in all layers[27]. When this information is added to that of the current study, it can be seen that local connections most closely follow the organizational plan of lateral corticocortical connections.

Other notable similarities between local and corticocortical connections include their patchy distributions. Like local connections, corticocortical connections may link a single column of cortex to many other discrete, spatially separated columns that correspond to the same visual field space[10,22]. With this in mind, the incomplete spatial coincidence in the distribution of retrograde and anterograde labeling from a single injection site may not be unique to local connections. Indeed, although most previous hodological studies employing a combination of anterograde and retrograde tracers[17,30,32,44,51,54] found evidence for corticocortical reciprocity, few of them were concerned with point-to-point correspondences between individual columns.

Some experimenters, however, have found evidence

for non-reciprocity upon examining their data at a finer spatial scale. Code and Winer examined the reciprocity of commisural connections in cat primary auditory cortex through use of a mixture of WGA–HRP and tritiated amino acids injected at a single locus[4]. As in the present study, the anterograde and retrograde labeling were found to have discontinuous and incompletely overlapping distributions. It was also noted, using the same methods, that complete reciprocity is lacking in the interconnections between the medial geniculate body and the auditory cortex in the rat[51]. Results from unpublished experiments in our laboratory indicate that the patchy connections linking areas 17 and 18 in the cat may not show complete reciprocity. In the macaque monkey also, connections between V2 (area 18) and V1 (area 17) have been suggested to be non-reciprocal at the columnar level[36]. Incomplete reciprocity at a fine spatial scale may thus characterize other reciprocal connections that are patchy in nature.

In the patches which were reciprocally connected with the injection site, retrogradely labeled cells sometimes appeared to be the targets of anterogradely labeled boutons. Single cells could thus make reciprocal circuits with one column of cortex, both receiving a projection from it, and projecting back to it. This type of circuit has also been shown for callosal projection cells in mouse motor cortex, some of which themselves receive synapses from callosally projecting cells[31].

The cell in Fig. 4 receives contacts from what is probably, as judged by its morphology, a basket cell axon[9, 37]. This type of negative feedback, if common, might make an important difference in the influence that activity in one column of cortex has on reciprocally compared to non-reciprocally connecting columns.

In the absence of physiological information on the functional properties of the injected column and those of the reciprocally and non-reciprocally connected columns, it is difficult to propose how, or even if, reciprocal and non-reciprocal connections function differently. What physiological properties are likely to be relevant to the reciprocity of local connections? As the spatial scale of these networks appears to be finer than the point spread function[7], receptive field location is not likely to be important. A column could project to several other columns and receive inputs from a different set of columns and yet, because of the smear in the visual space topography of the cortical map, both the input and output columns would have roughly the same visual space representation.

A parameter that has been shown to be important in the formation of both local and corticocortical patchy connections is the orientation preferences of connected columns. However, earlier studies have arrived at opposite conclusions with regards to whether the interconnections link together columns with similar[14] or different[27] orientation preference. While these discrepancies may be due to differences between: (1) long-range (> 1 mm) vs short-range (<1 mm) connections; (2) area 17 vs area 18; or (3) methodologies and tracers used[26], the fact remains that orientation selectivity and not ocular dominance appears to correlate with the patchy, local connections in visual cortex. Perhaps other parameters related to orientation selectivity, such as direction selectivity and end inhibition, require the non-reciprocal circuitry demonstrated in this report. These possibilities await further studies combining functional analysis of cortical columns with anatomical analysis of the reciprocity of patchy local circuits in visual cortex.

REFERENCES

1 Akers, R. and Killackey, H.P., Organization of corticocortical connections in the parietal cortex of the rat, *J. Comp. Neurol.*, 181 (1978) 513–538.

2 Boyd, J.D. and Matsubara, J.A., Reciprocal connections within cat visual cortex (area 18), *Soc. Neurosci. Abstr.*, 15 (1989) 799.

3 Cavada, C. and Goldman-Rakic, P.S., Posterior parietal cortex in rhesus monkey: II. Evidence for segregated corticocortical networks linking sensory and motor areas with the frontal lobe, *J. Comp. Neurol.*, 287 (1989) 422–445.

4 Code, R.A. and Winer, J.A., Columnar organization and reciprocity of commissural connections in cat primary auditory cortex (AI), *Hearing Res.*, 23 (1986) 205–222.

5 Colonnier, M., Synaptic patterns on different cell types in the different laminae of the cat visual cortex. An electron microscope study, *Brain Research*, 9 (1968) 268–287.

6 Coogan, T.A. and Burkhalter, A., Conserved patterns of cortico-cortical connections define areal hierarchy in rat visual cortex, *Exp. Brain Res.*, 80 (1990) 49–53.

7 Cynader, M.S., Swindale, N.V. and Matsubara, J.A., Functional topography in cat area 18, *J. Neurosci.*, 7 (1987) 1401–1413.

8 De Olmos, J. and Heimer, L., Mapping of collateral projections with the HRP-method, *Neurosci. Lett.*, 6 (1977) 107–114.

9 DeFelipe, J. and Fairen, A., A type of basket cell in superficial layers of the cat visual cortex. A golgi-electron microscope study, *Brain Research*, 244 (1982) 9–16.

10 Ferrer, J.M.R., Price, D.J. and Blakemore, C., The organization of corticocortical projections from area 17 to area 18 of the cats visual cortex, *Proc. R. Soc. Lond. Ser. B*, 223 (1989) 77–98.

11 Gabbott, P.L.A., Martin, K.A.C. and Whitteridge, D., Connections between pyramidal neurons in layer 5 of cat visual cortex (area 17), *J. Comp. Neurol.*, 259 (1987) 364–381.

12 Gerfen, C.R. and Sawchenko, P.E., An anterograde neuroanatomical tracing method that shows the detailed morphology of neurons, their axons and terminals: immunohistochemical localization of an axonally transported plant lectin, *Phaseolus vulgaris* leucoagglutin (PHA-L), *Brain Research*, 290 (1984) 219–238.

13 Gilbert, C.D. and Wiesel, T.N., Clustered intrinsic connections in cat visual cortex, *J. Neurosci.*, 3 (1983) 1116–1133.

14 Gilbert, C.D. and Wiesel, T.N., Columnar specificity of intrinsic horizontal and corticocortical connections in cat visual cortex, *J. Neurosci.*, 9 (1989) 2432–2442.

15 Horn, A.K.E. and Hoffman, K.-P., Combined GABA-immunocytochemistry and TMB–HRP histochemistry of pretectal nuclei projecting to the inferior olive in rats, cats and monkeys, *Brain Research,* 409 (1987) 133–138.
16 Huerta, M., Frankfurter, A. and Harting, J.K., Studies of the principal sensory and spinal trigeminal nuclei of the rat: projections of the superior colliculus, inferior olive, and cerebellum, *J. Comp. Neurol.,* 220 (1983) 147–167.
17 Irvine, D.R.F. and Brugge, J.F., Afferent and efferent connections between the claustrum and parietal association cortex in cat: a horseradish peroxidase and autoradiographic study, *Neurosci. Lett.,* 20 (1980) 5–10.
18 Itoh, K., Konishi, A., Nomura, S., Mizuno, N., Nakamura, Y. and Sugimoto, T., Application of coupled oxidation reaction to electron microscopic demonstration of horseradish peroxidase: cobalt-glucose oxidase method, *Brain Research,* 175 (1979) 341–346.
19 Jones, E.G., Coulter, J.D. and Hendry, S.H.C., Intracortical connectivity of architectonic fields in the somatic sensory, motor and parietal cortex of monkeys, *J. Comp. Neurol.,* 181 (1978) 291–348.
20 Kisvarday, Z.F., Martin, K.A.C., Freund, T.F., Magloczky, Z., Whitteridge, D. and Somogyi, P., Synaptic targets of HRP-filled layer III pyramidal cells in the cat striate cortex, *Exp. Brain Res.,* 64 (1986) 541–552.
21 Levay, S., Patchy intrinsic projections in visual cortex, area 18, of the cat: morphological and immunocytochemical evidence for an excitatory function, *J. Comp. Neurol.,* 269 (1988) 265–274.
22 Levay, S., The patchy intrinsic projections of visual cortex, *Progr. Brain Res.,* 75 (1988) 147–161.
23 Livingstone, M.S. and Hubel, D.H., Specificity of intrinsic connections in primary visual cortex, *J. Neurosci.,* 4 (1984) 2830–2835.
24 Luhmann, H.J., Millan, L.M. and Singer, W., Development of horizontal intrinsic connections in cat striate cortex, *Exp. Brain Res.,* 63 (1986) 443–448.
25 Martin, K.A.C. and Whitteridge, D., Form, function and intracortical projections of spiny neurons in the striate visual cortex of the cat, *J. Physiol.,* 353 (1984) 463–504.
26 Matsubara, J.A., Presence of GABA-immunoreactive neurons within intracortical patches in area 18 of the cat, in preparation.
27 Matsubara, J.A., Cynader, M.S. and Swindale, N.V., Anatomical properties and physiological correlates of the intrinsic connections in cat area 18, *J. Neurosci.,* 7 (1987) 1428–1446.
28 Maunsell, H.R. and Van Essen, D.C., The connections of the middle temporal visual area (MT) and their relationship to a cortical hierarchy, *J. Neurosci.,* 3 (1983) 2563–2586.
29 Mesulam, M.M., Tetramethylbenzidine for horseradish peroxidase neurochemistry, *J. Histochem. Cytochem.,* 26 (1978) 106–117.
30 Ogren, M. and Hendrickson, A., Pathways between striate cortex and subcortical regions in *Macaca mulatta* and *Saimiri sciureus*: evidence for a reciprocal pulvinar connection, *Exp. Neurol.,* 53 (1976) 780–800.
31 Porter, L.L. and White, E.L., Synaptic connections of callosal projection neurons in the vibrissal region of mouse primary motor cortex: an electron microscopic/horseradish peroxidase study, *J. Comp. Neurol.,* 248 (1986) 573–587.
32 Raczkowski, D. and Rosenquist, A.C., Connections of the multiple visual cortical areas with the lateral posterior-pulvinar complex and adjacent thalamic nuclei in the cat, *J. Neurosci.,* 3 (1983) 1912–1942.
33 Rockland, K.S., Intrinsic laminar lattice connections in primate visual cortex, *J. Comp. Neurol.,* 216 (1983) 303–318.
34 Rockland, K.S., A reticular pattern of intrinsic connections in primate area V2 (area 18), *J. Comp. Neurol.,* 235 (1985) 467–478.
35 Rockland, K.S. and Lund, J.S., Widespread periodic intrinsic connections in the tree shrew visual cortex, *Science,* 215 (1982) 1532–1534.
36 Rockland, K.S. and Virga, A., Terminal arborizations of individual 'feedback' axons projecting from area V2 to V1 in the macaque monkey: a study using immunocytochemistry of anterogradely transported *Phaseolus vulgaris*-leucoagglutinin, *J. Comp. Neurol.,* 285 (1989) 54–72.
37 Somogyi, P., Kisvarday, Z.F., Martin, K.A.C. and Whitteridge, D., Synaptic connections of morphologically identified and physiologically characterized large basket cells in the striate cortex of the cat, *Neuroscience,* 10 (1983) 261–294.
38 Sukekawa, K., Reciprocal connections between medial prefrontal cortex and lateral posterior nucleus in rats, *Brain Behav. Evol.,* 32 (1988) 246–251.
39 Symonds, L.L. and Kaas, J.H., Connections of the striate cortex in the prosimian, *Galago senegalensis, J. Comp. Neurol.,* 181 (1978) 477–512.
40 Symonds, L.L. and Rosenquist, A.C., Corticocortical connections among visual areas in the cat, *J. Comp. Neurol.,* 229 (1984) 1–38.
41 Symonds, L.L. and Rosenquist, A.C., Laminar origins of visual corticocortical connections in the cat, *J. Comp. Neurol.,* 229 (1984) 39–47.
42 Takeuchi, Y., Allen, G.V. and Hopkins, D.A., Transnuclear transport and axon collateral projections of the mamillary nuclei in the rat, *Brain Res. Bull.,* 14 (1985) 453–468.
43 Tigges, J., Spatz, W.B. and Tigges, M., Reciprocal point to point connections between parastriate and striate cortex in the squirrel monkey (*Saimiri*), *J. Comp. Neurol.,* 148 (1973) 481–490.
44 Tigges, J., Tigges, M. and Perachio, A.A., Complementary laminar terminations of afferents to area 17 originating in area 18 and in the lateral geniculate nucleus in squirrel monkeys, *J. Comp. Neurol.,* 176 (1977) 87–100.
45 Torrealba, F., Olavarria, J. and Carrasco, M.A., Cortical connections of the anteromedial extrastriate visual cortex in the rat, *Exp. Brain Res.,* 56 (1984) 543–549.
46 Trudrung, P. and Schumacher, U., Analysis of wheat germ agglutinin (WGA), *Phaseolus vulgaris* (PHA-L), and Lens *Culinaris agglutinin* (LCA) binding to isolated rat neocortical membrane glycoproteins and to brain tissue sections, *Brain Research,* 497 (1989) 399–401.
47 Tusa, R.J., Rosenquist, A.C. and Palmer, L.A., Retinotopic organization of areas 18 and 19 in the cat, *J. Comp. Neurol.,* 185 (1979) 657–678.
48 Vogt, B.A. and Pandya, D.N., Cortico-cortical connections of somatic sensory cortex (areas 3, 1 and 2) in the rhesus monkey, *J. Comp. Neurol.,* 177 (1978) 179–192.
49 Vogt, B.A., Sikes, R.W., Swadlow, H.A. and Weyland, T.G., Rabbit cingulate cortex: cytoarchitecture, physiological border with visual cortex, and afferent cortical connections of visual, motor, postsubicular, and intracingulate origin, *J. Comp. Neurol.,* 248 (1986) 74–94.
50 White, E.L. and DeAmicis, R.A., Afferent and efferent projections of the region in mouse SmI cortex which contains the posteromedial barrel subfield, *J. Comp. Neurol.,* 175 (1977) 455–482.
51 Winer, J.A. and Larue, D.T., Patterns of reciprocity in auditory thalamocortical and corticothalamic connections: study with horseradish peroxidase and autoradiographic methods in the rat medial geniculate body, *J. Comp. Neurol.,* 257 (1987) 282–315.
52 Winguth, S.D. and Winer, J.A., Corticocortical connections of cat primary auditory cortex (AI): laminar organization and identification of supragranular neurons projecting to area AII, *J. Comp. Neurol.,* 248 (1986) 36–56.
53 Wise, S.P. and Jones, E.G., Cells of origin and terminal distribution of descending projections of the rat somatic sensory cortex, *J. Comp. Neurol.,* 175 (1977) 129–158.
54 Wong-Riley, M., Reciprocal connections between striate and prestriate cortex in squirrel monkey as demonstrated by combined peroxidase histochemistry and autoradiography, *Brain Research,* 147 (1978) 159–164.
55 Wouterlood, F.G. and Groenewegen, H.J., Neuroanatomical tracing by use of *Phaseolus vulgaris*-leucoagglutinin (PHA-L): electron microscopy of PHA-L filled neuronal somata, dendrites, axons, and axon terminals, *Brain Research,* 326 (1985) 188–191.
56 Zeki, S. and Shipp, S., The functional logic of cortical connections, *Nature,* 335 (1988) 311–317.