Inferior Parietal Lobule Projections to the Presubiculum and Neighboring Ventromedial Temporal Cortical Areas

SONG-LIN DING,1* GARY VAN HOESEN,1,2 AND KATHLEEN S. ROCKLAND1
1Department of Neurology, University of Iowa, Iowa City, Iowa 52242
2Department of Anatomy and Cell Biology, University of Iowa, Iowa City, Iowa 52242

ABSTRACT

The entorhinal and perirhinal cortices have long been accorded a special role in the communications between neocortical areas and the hippocampal formation. Less attention has been paid to the presubiculum, which, however, is also a component of the parahippocampal gyrus, receives dense inputs from several cortical areas, and itself is a major source of connections to the entorhinal cortex (EC). In part of a closer investigation of corticohippocampal systems, the authors applied single-axon analysis to the connections from the inferior parietal lobule (IPL) to the presubiculum. One major result from this approach was the finding that many of these axons (at least 10 of 14) branch beyond the presubiculum. For 4 axons, branches were followed to area TF and to the border between the perirhinal and entorhinal cortices, raising the suggestion that these areas, which sometimes are viewed as serial stages, are tightly interconnected. In addition, the current data identify several features of presubicular organization that may be relevant to its functional role in visuospatial or memory processes: 1) Terminations from the IPL, as previously reported for prefrontal connections (Goldman-Rakic et al. 1984 Neuroscience 12:719–743), form two to four patches in the superficial layers. These align in stripes, but only for short distances (~1.5 mm). This pattern suggests a strong compartmentalization in layers I and II that is also indicated by cytochrome oxidase and other markers. 2) Connections tend to be bistriated, terminating in layers I–II and deeper in layer III. 3) Single axons terminate in layer I alone or in different combinations of layers. This may imply some heterogeneity of subtypes. 4) Individual axons, both ipsilateral projecting (n = 14 axons) and contralateral projecting (n = 6 axons), tend to have large arbors (0.3–0.8 mm across). Finally, the authors observe that projections from the IPL, except for its anteriormost portion, converge at the perirhinal-entorhinal border around the posterior tip of the rhinal sulcus. These projections partially overlap with projections from ventromedial areas TE and TF, and this convergence may contribute to the severe deficits in visual recognition memory resulting from ablations of rhinal cortex. J. Comp. Neurol. 425: 510–530, 2000.

The presubiculum is a multilayered, periallocortical structure located between the subiculum and the parasubiculum that is often considered part of the hippocampal formation. It receives connections from several cortical regions, including area 7a of the inferior parietal lobule (Seltzer and Van Hoesen, 1979; Seltzer and Pandya, 1984; Cavada and Goldman-Rakic, 1989), as well as areas in the prefrontal (Goldman-Rakic et al., 1984; Selemon and Goldman-Rakic, 1988; Morris et al., 1999) and cingulate cortices (Pandya et al., 1981) and the superior temporal gyrus (Amaral et al., 1983). Because a major efferent outflow from the presubiculum is to layer III of the entorhinal cortex (EC), these cortical inputs are important as potential influences on the entorhinal-hippocampal component of the perforant pathway, including projections to

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*Correspondence to: Song-Lin Ding, M.D., Department of Neurology, University of Iowa, 200 Hawkins Drive, Iowa City, IA 52242-1053. E-mail: slding@blue.weeg.uiowa.edu

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the subiculum. They can be viewed as critical nontemporal corticohippocampal systems.

Recently, the presubiculum has attracted renewed interest because of the physiological demonstration in both rodents and monkeys (Taube et al., 1996; Robertson et al., 1999) that some neurons in this structure selectively increase their discharge rate when the subject’s head is pointed, compass-like, in a best direction for that cell. The occurrence of “head-direction” cells bespeaks a role for the presubiculum in visuospatial processes. This would be compatible with the input to this region from the inferior parietal lobule (IPL), which has long been associated with visuospatial functions. It thus seemed important to further investigate the functional architecture of the presubiculum, with particular attention to the organization of the connections from the IPL.

The present study used single axon analysis to visualize and analyze the microarchitecture of parietal connections to the presubiculum. In particular, we were interested in establishing the size, configuration, and laminar termination of individual arbors and determining whether these might exhibit any features distinct from terminations in sensory cortical areas. Our results indicate that some parietal axons terminating in the presubiculum have relatively large arbors that often are distributed in distinct patches. Another finding was that many of these axons send branches to area TF and to the lateralmost border of the EC with the perirhinal cortex (PRh). This result was unexpected, because the presubiculum and area TF are generally viewed as serial stages in the corticohippocampal pathways. It implies that these “two” pathways can function as a tightly interconnected network.

**MATERIALS AND METHODS**

Tracer injections of biotinylated dextran amine (BDA) were placed in different portions of the inferior parietal lobule in two male (weight, 4 kg and 11.5 kg) and five female (weight, 4.6–6.6 kg) rhesus monkeys (*Macaca mulatta*). Animals were tranquilized with ketamine (11 mg/kg, i.m.) and were maintained under deep anesthesia with Nembutal (25 mg/kg, i.v.). Surgery was performed on anesthetized animals under sterile conditions and according to institutional and U.S. Department of Agriculture guidelines, as specified in approved Animal Care and Use Forms (University of Iowa). Cortical areas of interest were localized by direct visualization, subsequent to craniotomy and durotomy, in relation to sulcal landmarks (i.e., the intraparietal and superior temporal sulci).

One to four pressure injections were made of BDA [10% in 0.0125 M phosphate-buffered saline (PBS), pH 7.2]. Equal parts of 10,000 and 3,000 molecular weight BDA (Molecular Probes, Eugene, OR) were used. Injections were deliberately made large to cover significant portions of a given cortical field and to optimize labeling of fine-caliber axons (see Table 1, Fig. 1). Multiple injections were placed close together so that they would merge into a single injection site. Animals were given postsurgical doses of antibiotic and analgesics, allowed to recover, and survived for 18–29 days. They were then reanesthetized with Nembutal (75 mg/kg) and perfused transcardially, in sequence, with 0.9% saline and 0.5% sodium nitrite; 4%
Fig. 2. Photomicrographs showing laminar organization of the presubiculum. A: Thionin stain for cell bodies. Asterisk indicates cell sparse lamina dissecans. B: Gallyas stain for myelin. A and B are from closely adjacent sections, and arrowheads point to corresponding blood vessels. C: Immunocytochemistry (icc) for parvalbumin (Parv). D: Icc for calbindin (CB). C and D are from adjacent sections, and arrowheads point to corresponding features. E,F: Higher magnification photomicrographs of closely adjacent sections reacted for Parv (E) and for CB (F). G: Section reacted for cytochrome oxidase. Patches are evident in layer I and in the superficial part of layer II. PaS, parasubiculum; L, lateral (edge of the presubiculum). Scale bars = 500 µm in A (also applies to B), C (also applies to D), G; 100 µm in E (also applies to F).
paraformaldehyde; and chilled 0.1 M phosphate buffer with 10%, 20%, and 30% sucrose. For two of the animals (P5 and P7), 0.25% glutaraldehyde was added to the fixative.

Brains were cut serially in the coronal plane by frozen microtomy (at 50 μm thickness) and processed histologically for BDA (Brandt and Apkarian, 1992; Veenman et al., 1992). Tissue was reacted for 20–24 hours in avidin-biotin complex (ABC) solution (Vector Laboratories, Burlingame, CA) at room temperature (ABC Elite kits; 1 drop of reagents per 7 ml of 0.1 M PBS). In the final step, BDA was demonstrated by 3,3′-diaminobenzidine tetrahydrochloride (DAB) histochemistry with the addition of 0.5% nickel-ammonium sulfate.

Analysis

This study emphasizes serial section reconstruction of single axons terminating in the presubiculum. Recon-
structions were carried out with the aid of a camera lucida microscope attachment (BH-2 microscope; Olympus, Tokyo, Japan). Axons were drawn on paper at magnifications of $\times 100$ and $\times 600$ (oil-immersion). Complex portions of the axon (i.e., branch points or crowded fields) were drawn and/or verified under oil immersion at $\times 1,000$ magnification. The overall projection focus in each case was also charted for extent, pattern, and laminar organization.

The presubiculum and entorhinal cortex were readily identified by their specialized lamination. This was visualized by light cellular background staining from DAB and/or Nomarsky optics. Nissl counterstains were only occasionally used, as they tended to camouflage fine axonal processes and preclude any further reconstructions. Other fields, including the localization of injection sites within the IPL, were identified by architectonic features (in counterstained material), by overall connectivity patterns, and by comparison with published mapping studies. The series of injections in the IPL was designed to cover this region in both the mediolateral (ML) and anteroposterior (AP) axes (except for the most posterior sector, which has been considered as a component of the occipital

Fig. 4. Photomicrographs showing bistratified distribution of terminal labeling. A: Terminations are concentrated in layers I and upper III (from case 9). A few neurons retrogradely filled from the biotinylated dextran amine (BDA) injection are evident in layer III at the left. B: Three distinct terminal patches are evident in layers I and II (from case 9). C: Higher magnification view of the section shown in B. Arrows in A and B point to terminal patches. Scale bars = 100 $\mu$m in A,C; 200 $\mu$m in B.
lobe). The IPL, as shown by architectonic and connec-
tional studies (see, e.g., Pandya and Seltzer, 1982), en-
compasses multiple smaller areas. For ease of descrip-
tion, in this study we distinguish only between the
posterior and anterior subregions, areas 7a and 7b,
which are associated, respectively, with visual and so-
matosensory processes.

The technique of single axon reconstruction has been used
extensively over the last 10 years in work from this and
other laboratories (see, e.g., Saleem and Tanaka, 1996).
However, it may be useful to recapitulate some of the key
features of the procedure. An important first step, because
extracellular injections result in the labeling of many thou-
sands of axons, is the selection of individual processes ("pro-

Fig. 5. Photomicrographs of the border between the perirhinal
(PRh) and entorhinal (ERh) cortices (arrow in A) at the posterior tip
of the rhinal sulcus (RS). A: Thionin stain for cell bodies. B: Terminal
labeling (from case 9) in the depth of the rhinal sulcus (RS) at the
border between the PRh (right) and ERh (left) cortices. Arrowhead
points to an isolated branch from a single axon. C: Single axon (from
case 9) terminating in layer II/III of the medial bank of the RS.

(The lateral surface is at the top and is rotated 90° from A and B).
D: Higher magnification of C (arrows point to corresponding features).
E: Higher magnification of D (arrowheads point to corresponding
features). Serial reconstruction of this axon is shown in Figure 19. L,
lateral. Scale bars = 100 μm in A; 200 μm in B,C; 50 μm in D; 20 μm
in E.)
files") for detailed analysis. Most efficient, in our experience, has been to identify well-isolated arboris in the gray matter and then to follow the axon trunk back into the white matter and toward its origin in the injection site. The selected profile is followed through sequential sections. This is accomplished first by matching at low magnification (×40) a set of “landmarks,” blood vessels and 4–8 axon segments, possibly but not necessarily including the selected profile. Once the general region of interest is confirmed between neighboring slides, the matching process is repeated at higher magnification (typically ×200 or ×400 for gray matter and ×100 for white matter). Part of the matching process includes matching segments in the Z-dimension, at the top and bottom surfaces of the section. The segment of interest is drawn in, as well as the landmarks, and the process repeated for the next pair of adjoining slides. The set of landmarks is usually not constant over the course of the reconstruction, and the landmarks are not drawn in the final summary diagram.

Because serial section reconstruction is labor intensive, the sample size tends to be relatively small. Therefore, to achieve standardization, we prefer to concentrate on only two or three brains for the reconstructions. Features identified by this technique, however, to some extent, can be confirmed by reconstructions through shorter series and/or by scanning through individual sections; and additional brains in an experimental group are used for this purpose.

Two cases with injections of Phaseolus vulgaris-leucoagglutinin (PHA-L) into ventromedial area TE and area TF were used for comparison with the pattern of

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Fig. 6. Darkfield photomicrograph of terminations in the presubiculum anterogradely labeled by injections into ventromedial area TE (in the lateral bank of the occipitotemporal sulcus (OTS)). Arrowheads point to some terminations. Scale bar = 100 μm.

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Fig. 7. Photomicrographs of terminations at the posterior tip of the RS labeled by injections into ventromedial area TE (in the lateral bank of the OTS). A: Low-magnification view of the border between the PRh (arrowhead) and ERh (single arrow) cortices. B,C: Higher magnification of label in the PRh cortex in the upper (arrowhead) and deeper (double arrow) layers. D: Higher magnification of label medi ally in the upper layers of the ERh cortex (arrows point to corresponding features in A and D). M, medial. Scale bars = 500 μm in A; 100 μm in B–D.
parietal connections to the presubiculum and associated fields. Some of the results from these cases have been reported in Wellman and Rockland (1997) and Rockland and Van Hoesen (1999). Several series of normal brains that were treated for parvalbumin, calbindin, or cytochrome oxidase (CO) were also consulted for architectonic features and boundaries. Preliminary results were presented in abstract form (Ding et al., 1999).

**RESULTS**

**Laminar structure of the presubiculum**

The presubiculum is a stripe-like periallocortical zone, about 2.0 mm wide × 6.0 mm long, immediately bordering the subiculum at the medial edge of the temporal lobe. It is easily identified by staining for cell bodies, myelin, or various standard immunohistochemical markers (Fig. 2).

In Nissl stains (Fig. 2A), the presubiculum has four distinct strata: a thin, cell-sparse layer (layer I); a broad, cell-dense layer (layers II–III); another cell-sparse zone (layer IV or lamina dissecans); and a deeper zone of more loosely packed neurons (layers V–VI). The superficial cell-dense layer is further divisible, with a thin outermost layer (layer II) of more tightly packed neurons. Layer I is about 150 µm thick; layers II–III are about 400 µm thick (including the outermost sublayer, which measures about 100 µm); and the deeper layers are about 500 µm wide. Laminar dimensions, however, are highly variable, especially over the ML extent of the presubiculum. All of the layers are thinner at the lateral border with the subiculum and thicker at the medial edge, which usually wraps over the medial lip of the parahippocampal gyrus.

In tissue reacted for parvalbumin, there is a dense band of parvalbumin-positive neuropil and cell bodies corresponding to layers II–III. The calbindin pattern is slightly different and more complex. There is a dense, calbindin-positive plexus in layer I; a calbindin-poor band corresponding to the cell dense layer II of Nissl stains; and another band of calbindin-positive neuropil, with a few cell bodies, in layer III. Thus, the calbindin- and parvalbumin-positive bands are complementary in layers I (calbindin positive and parvalbumin negative) and II (calbindin negative and parvalbumin positive) but overlap in layer III (Fig. 2C–F). Many calbindin-positive fibers and a few parvalbumin-positive fibers are also discernible in the white matter below the presubiculum.

In addition to the main features of laminar organization, histochemistry for CO revealed sporadic patches, about 300 µm wide, in layer I (Fig. 2G). The occurrence of patches varied between different animals and for the same animal across different AP levels of the presubiculum.

Layer I is further distinguished by a thick plexus of myelinated fibers (the reticular substance of Arnold; Gloor, 1997), which is especially prominent at posterior and middle levels of the presubiculum. These fibers may correspond to corticocortical connections, some of which have long preterminal portions in layer I (see below). Alternately or additionally, they may correspond to thalamic inputs from the anterior nucleus or pulvinar. No periodicity was discernible in myelin preparations (Fig. 2B).

**Global pattern of parietal projections to the presubiculum**

In agreement with previous reports (Seltzer and Van Hoesen, 1979; Seltzer and Pandya, 1984; Selemon and Goldman-Rakic, 1988; Cavada and Goldman-Rakic, 1989), dense connections to the presubiculum were found from the middle and posterior subdivisions, but not from the more anterior subdivisions, of the IPL (area 7b; Table 1, 1

1In an alternate nomenclature (Lorente de No, 1934; Rosene and Van Hoesen, 1987), the superficial layers of the presubiculum (II and III) are termed the lamina principalis externa, and the deeper layers (V and VI) are called the lamina principalis interna. These are separated by the cell-poor lamina dissecans. This nomenclature, however, is less convenient for designating subdivisions within the superficial layers and, thus, has not been used in this report.
Figs. 1, 3, 4). In the presubiculum, with our distribution of injection sites, connections were dense in the posterior one-third and moderate in its middle portion, with no projections to the anterior one-third. More anterior injections in the IPL tended to produce labeling in posterior regions of the presubiculum, and posterior injections resulted in terminations located more anteriorly. At their most dense, projection foci tended to cover almost the whole ML extent of the presubiculum. In all cases, terminations concentrated in layers I and III, were somewhat less dense in layer II and were only sparsely distributed in the deeper layers (IV–VI). Terminations tended to have a bistratified distribution. In layers I–II, they frequently formed two to four patches about 0.15–0.30 mm across and separated by 0.4–0.6 mm from center to center (Fig. 4). These aligned in stripes over short distances (~1.5 mm AP); however, in reconstructions of serial sections, the stripes appeared to merge and fragment in an overall complicated pattern. Terminations in layer III often appeared patchy as well, but the pattern was less distinct than in layers I–II. Patches were not sharply delimited, as some terminations occurred in the interpatch spaces (Fig. 4). The superficial system of patches may correspond to or overlap with the pattern of CO staining, but further experiments with double labels will be necessary to determine the exact relationship. In the medially adjoining parasubiculum (see Table 1), there were moderate projections from the five posterior injection sites (cases 5–9).

Global connections to areas TE and TF

Regions around the occipitotemporal sulcus (OTS), as reported by other studies (Seltzer and Pandya, 1984; Cavada and Goldman-Rakic, 1989; Suzuki and Amaral, 1994a; Webster et al., 1994), received projections from all except the anteriormost injection (case 11). Terminations formed several foci in the lateral or medial banks of the OTS or medial to the sulcus. The medial foci correspond to area TF, as classically defined (Von Bonin and Bailey, 1947), and the lateral foci correspond to area TEO posteriorly or, more anteriorly, to a region characterized as ventromedial TE (Rockland and Van Hoesen, 1994; see also Felleman et al., 1997). In area TEO, terminations were mainly in layers I and VI; in the anterior areas, terminations in the dense core of the projection tended to be columnar.

Global connections to the rhinal sulcus

All seven injections resulted in some terminations in the vicinity of the rhinal sulcus (RS); however, for the most
anterior injection (case 11), these were very light (Table 1, Fig. 5). Despite the different locations of the injections within the IPL, most projections converged at a common locus, between the anterior OTS and the posterior tip of the RS and in the depth of the posterior RS (projections in case 10 extended somewhat more anterior). This is a complicated transition zone at the border between the perirhinal and entorhinal cortices that has been separately designated as the “prorhinal cortex” (Van Hoesen and Pandya, 1975). In recent studies (Amaral et al., 1987; Saleem and Tanaka, 1996), the medial bank of the RS has been included within the entorhinal cortex (EC). Terminations directly targeted this region, extending about 2.0 mm from the sulcal depth along both the lateral wall and the medial wall. In cases 7, 9, and 10, projections continued farther anterior in the RS and, at these levels, extended farther into the EC toward the medial lip of the RS. Terminations in the medial bank of the RS occurred in layers I–III. Sparse connections were observed in the contralateral parasubiculum (Table 1) in case 7 and (even sparser) in case 8.

In the contralateral RS, the more anterior cases (cases 9, 11), in which ipsilateral connections were relatively lighter, had no discernible terminations. Cases 6 and 8 had sparse projections, although these were less dense and less extensive than those on the side ipsilateral to the injections. Case 7 had abundant fibers in the white matter approaching the posterior EC (more anterior sections were not available; Table 1).

Temporal connections to the presubiculum and RS

Of the two cases available with injections in temporal regions, ventromedial TE, but not TF, sent some projections to the presubiculum (Fig. 6). These were less extensive (<2.0 mm AP) and less dense than projections from parietal areas (contralateral hemispheres were not available).

Both temporal cases resulted in projections to the RS. Projections from ventromedial TE terminated mainly at the lateral border of the EC, extending about 2.0 mm into the medial bank (Fig. 7). This is the same border zone targeted by the projections from the IPL but is more anterior. Projections from area TF similarly targeted the lateral edge of the EC, but extended farther medially. The laminar pattern was similar to the parietal connections, except that it was denser in layer I.

**Contralateral connections to the presubiculum and RS**

Five of the contralateral hemispheres were available for inspection (Table 1). Of these, the two most anterior injections (cases 9, 11) did not result in any detectable label in the presubiculum. Case 6, in which the injection site was posterior, had connections of moderate density in the contralateral presubiculum (extending 0.35 mm AP). In case 7, with a larger injection situated in the middle portion of the IPL, the contralateral connections were more extensive (4.5 mm AP). Like the ipsilateral connections, these homotypical commissural terminations were concentrated in layers I–III. Sparse connections were observed in the contralateral parasubiculum (Table 1) in case 7 and (even sparser) in case 8.

**Fig. 10.** Camera lucida reconstruction of axon 7-1 (case 7). A: Low-magnification schematic view showing that this axon terminates with a single arbor located within one of the projection patches. B: The patch is indicated by small dashed lines in this outline of section 518. C: Higher magnification detail of terminal specializations. Arrows in A and C point to corresponding features; the arrow in B localizes the axon trunk in relation to the projection patch. The numbered arrows in A indicate how far the main axon was followed in both directions in the white matter. hf, Hippocampal fissure; dg, dentate gyrus; sub, subiculum.
Connections to CA1

On the basis of three of these cases (cases 5, 7, and 9), direct connections had been previously reported from the IPL to CA1 (Rockland and Van Hoesen, 1999). Direct connections to CA1 were also found in three of the additional four cases reported here (Table 1, Fig. 8). Connections were most dense in cases with more posterior injections (in area 7a). In case 11 (situated in area 7b) and case 10 (anterior in area 7a), terminations were very light and were restricted to the most posterior part of CA1.

Single axons terminating in layer I of the presubiculum

Long axon fragments (>0.5 mm) could easily be found in layer I in single sections (Fig. 3). The finding that some axons target exclusively layer I was more precisely dem-
onstrated by serial reconstruction of four axons (case 5), three of which are shown in Figure 9. The total lengths of these axons in layer I were 1.2 mm, 2.0 mm, and 3.0 mm. Some boutons were scattered along the full portion of the axon in layer I, but most were concentrated at the distal 0.5–0.7 mm.

**Single axons terminating in the presubiculum with simple arbors**

Other axons gave rise to simple arborizations, which extended into layers I–III of the presubiculum. Five of these axons were reconstructed, three of which are shown in Figures 10–12. For axon 7-1, there was a single arbor at least 0.4–0.5 mm wide (fine, distalmost portions were lost in a patch of dense terminations) (Fig. 10).

Axon 5-6 also had a single projection focus in the presubiculum. For this axon, like 7-1, after giving off terminations in the presubiculum, the main axon continued ventral and lateral in the white matter. Three branches were followed to projection foci in a region in the medial bank of the OTS that probably corresponds to area TF. A fourth anterior branch was followed to the border of entorhinal and perirhinal cortices (Fig. 11).

Axon 5-3 had two separate arbors, one lateral and slightly anterior to the other. Both had terminations in layers I–III (Fig. 12). The medial arbor measured about 0.8 mm ML × 0.9 mm AP, and the lateral arbor (at the curved arrow in Fig. 12) measured 0.8 mm ML × 0.3 mm AP. This axon in addition sent branches to three foci in area TF (separated by intervals of 1.0–1.5 mm) and three
Fig. 13. Camera lucida reconstruction (through 261 sections) of axon 5-2 with a complex arborization extending over a large mediolateral extent of the presubiculum. This consists of two large arbors (redrawn at higher magnification at the open arrow and the left-pointing arrow); a smaller, medially located arbor (at the right-pointing arrow) and five partially overlapping, smaller arbors (unmarked; see also the photomicrographs in Fig. 15C–E). Each of these arbors was associated with a denser projection patch, as indicated by the icons within the small dashed lines on the outlines of sections 474, 489, and 491. Note that several of the arbors descend from more superficial branches into the deeper layers. This axon continues in the white matter beyond the presubiculum (and was followed through section 588). The proximal portion of this axon was followed to section 314 (arrow).
foci at the lateral edge of the EC (separated by intervals of 1.0 mm: at sections 602, 619, 640, and 657). Within these foci, the actual terminations were either too fine to trace (in the EC) or were lost in a field of dense terminations (in area TF).

Single axons terminating in the presubiculum with complex arborizations

Five axons were documented with multifocal, more complex arborizations, of which three are illustrated and described below: Axon 5-2 had two large arbors (1.0 mm ML × 0.7 mm AP and 0.6 mm ML × 0.6 mm AP) and multiple smaller arbors (Fig. 13). Terminations were in layers I–III. Five smaller arbors partially overlapped in two projection patches that were separated by 1.0 mm AP (sections 457–460 and 479–483). A sixth arbor was situated about 0.8 mm laterally. Overall, the arbors contributed to four patches distributed over 2.4 mm ML × 1.5 mm AP. Beyond the presubiculum, the axon trunk continued ventrally and laterally to the white matter below area TF.

Axon 7-4 had five distinct branches that partially converged in three termination patches distributed over 1.5 mm ML × 2.0 mm AP. This axon did not send collaterals beyond the presubiculum (Fig. 14A).

Axon 7-5 had multiple branches directed to three termination patches spaced over 1.0 mm ML × 1.5 mm AP (terminations for the mediallymost branch could not be followed). Collaterals were traced to several foci in area TF and to the lateral edge of the EC (Fig. 14B).

In addition to the more complete reconstructions described above, measurements were made of six, partially reconstructed single arbors from cases 5, 8, and 10 (Fig. 15). These ranged from 0.4 mm to 0.5 mm in the longest dimension.

Single axons terminating in the contralateral presubiculum

Of six axons that were analyzed in the contralateral hemisphere from case 7, four had simple arborizations, of which three are illustrated (Figs. 16–18). Arbors were concentrated in layers I–III. Two of these axons (C1 and C2) had a single arbor. One measured 0.6 mm ML × 0.8 mm AP, and the other measured about 0.6 mm in diameter (Fig. 16).

Axon C-3 had three arbors, all targeting one projection patch. There was one large, principal arbor (about 0.6 mm in diameter; straight arrows in Fig. 17); a small extension (~0.2 mm in diameter); and a second small arbor (0.2 mm in diameter; curved arrow in Fig. 17). The second arbor was offset from the main cluster by an interval of about 1.0 mm.

Axon C-4 had a complex arborization. There was one principal arbor (X at 355 in Fig. 18) and several other branches
that converged in a second focus. The two foci were separated by 1.2 mm ML but were at about the same AP plane.

Of these six axons, axons C-1 and C-4 (and one that is not illustrated) had collaterals that clearly continued in the white matter beyond the presubiculum. Axons C-1 and C-3 were followed only a short distance into the white matter, and the presence or absence of collaterals could not be ascertained.

Fig. 15. Photomicrographs from case 5 of portions of single BDA-labeled arbors in the presubiculum. A: Arbor terminating in layer III. B: Higher magnification of the area indicated by the arrow in A. C: Arbor terminating in layers II–III. This arbor is part of an axon that is illustrated more fully in Figure 13 (at the open arrow). D,E: Progressively higher magnifications of the area indicated by the arrow in C (D) and D (E). F: Arbor terminating in layers I–II. G: Higher magnification of the area indicated by the arrow in F. Scale bars = 200 μm in A,C,F; 100 μm in D; 50 μm in B,E,G.
Single axons terminating in RS

One axon (from case 9) was partially reconstructed at its distal termination at the lateral border of the EC. There were two partially overlapping arbors, about 0.30 mm and 0.45 mm wide each (Figs. 5C, 19). Together, these arbors extended over four cell islands in layer II with some incursion, medially, in the subjacent layer III. Terminations occurred in both the cell islands and the interisland spaces and also deeper in layer III along the ascending portion of the axon. A total of 263 boutons were counted along this segment. Two other single arbors (cases 9 and 10) were partially reconstructed and were found to measure about 0.30 mm wide.

Axon caliber and bouton counts

Observations were made on labeled axon segments in the white matter below the presubiculum. These varied in diameter. Most were about 1.0 \( \mu \text{m} \) wide, a few were large (2.0–3.0 \( \mu \text{m} \) wide), and many appeared to be \(<1.0 \mu \text{m}\).

Axon 5-2 had a total of 2,668 boutons (738 and 1,559 in the two large arbors and 371 among the six smaller arbors). Of the three contralateral arbors illustrated, axon C-1 had at least 400 boutons, axon C-2 had 1,545 boutons, and axon C-3 had 931 boutons. Bouton numbers in layers I–II were difficult to assess because of the overall density of the label. These numbers might be somewhat low because of the possibility of further fine distal extensions. Boutons were a mix of stalked (terminaux) and beaded (en passant) and were not qualitatively distinguishable from typical corticocortical terminations.

DISCUSSION

Connections to the presubiculum from area 7a of the IPL have been reported in previous studies using other
techniques (Seltzer and Van Hoesen, 1979; Seltzer and Pandya, 1984; Cavada and Goldman-Rakic, 1989). By using single axon reconstruction, the present work further demonstrates that 1) individual arbors terminating in the presubiculum are relatively large, and 2) individual axons commonly send branches to other targets in the parahippocampal region (area TF, perirhinal cortex, and entorhinal cortex) in addition to the presubiculum. These findings are discussed in relation to the functional architecture of the presubiculum and their possible implications for multiple memory systems associated with the temporal lobe.

Fig. 17. Camera lucida reconstruction of axon C-3 (case 7) terminating in the presubiculum contralateral to the injection site. This axon has one large arbor (about 0.6 mm in diameter; solid straight arrow) and a smaller arbor (solid curved arrow). These are offset by about 1.0 mm AP and 1.0 mm ML. The larger arbor itself has two partially overlapping components (open arrow) and a small posterior extension. The position of the axon is indicated at one AP level by the solid straight arrow and the small dashed lines on the outline of section 317. These represent a dense projection patch.
Configuration of individual arbors

There are still relatively few reports about corticocortical connections at the level of single axons. The available evidence suggests that, with the exception of feedback and other inputs to layer I, individual arbors tend to be ≤0.3 mm in diameter; for example, terminations from area TE to perirhinal cortex (Saleem and Tanaka, 1996) and terminations from several visual cortical areas (summarized in Rockland, 1997). By comparison, terminations from the IPL to the presubiculum cover larger territories (0.3–0.8 mm) either as single or multiple arbors (Fig. 20). Given the narrow width of the presubiculum, the large terminal fields are even more substantial. Possibly, the size of IPL arbors may be less related to their termination in the presubiculum than to their origin in the IPL; but preliminary results indicate that arbors terminating in area TF are ≤0.3 mm in size. This observation is consistent with what appears to be a trend associated with limbic-type fields. That is, other studies have reported divergent connections within both the hippocampal (Sik et al., 1993) and entorhinal (Tamamaki and Nojyo, 1993; Wellman and Rockland, 1997) pathways.

Another system of divergent terminations that may serve as a useful comparison is the subpopulation of magnocellular connections from the lateral geniculate nucleus to the primary visual cortex. These axons have large arbors (0.3–0.5 mm × 0.6–1.2 mm), large numbers of boutons (>3,200), and contact large numbers of postsynaptic neurons (Blasdel and Lund, 1983; Freund et al., 1989). In the case of magnocellular axons, these features correlate with high temporal sensitivity and a role in motion detection. In the case of the presubiculum, large terminal fields, which presumably contact large postsynaptic populations, might be consistent with a posited involvement of this structure in visuospatial functions, such as path integration or navigation (see, e.g., Robertson et al., 1999).

Some axons from the IPL selectively target layer I, where they can extend for distances of 1.0–3.0 mm. This pattern is reminiscent of “feedback” connections in the early sensory pathways. Like sensory feedback connections, layer I terminations might originate from neurons in layer 6 and may well be a distinct subpopulation. Whether they are functionally similar to “classical” feedback connections is less apparent (for further discussion of problems in classification resulting from over-reliance on laminar patterns, see Wellman and Rockland, 1997).
commonly form a bistratified pattern. This laminar com-

Fig. 20. Schematic diagram summarizing the different configura-

Banding pattern of terminations
Goldman-Rakic et al. (1984), in their earlier autoradi-

Functional implications
The recent demonstration of “head-direction cells” in

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visuospatial processes is well known (Burgess et al., 1997). Further experiments will be necessary, however, to determine the structural and functional interactions of these connections with the dense inputs received by the presubiculum from other regions, including prefrontal (Goldman-Rakic et al., 1984; Selemon and Goldman-Rakic, 1988), cingulate (Pandya et al., 1981), and superior temporal (Amaral et al., 1983) cortices and subcortical centers, such as the anterior thalamus, basal amygdaloid nucleus, claustrum, and medial pulvinar (Shipley and Sorensen, 1975; Niimi, 1978; Krayniak et al., 1979; Aggleton, 1986; Amaral and Insausti, 1990).

The hippocampal formation and neocortical areas, as remarked in other studies (Goldman-Rakic et al., 1984), are interlinked by multiple channels. Our results emphasize that the posterior IPL has at least three moderately direct levels of access to the hippocampal formation (Fig. 20). Most direct are 1) the projections terminating in CA1 (Rockland and Van Hoesen, 1999; this report) and 2) those terminating in the perirhinal cortex and adjoining EC. In addition, there are 3) abundant indirect pathways to the EC from parietal-recipient regions in area TF and the presubiculum (Van Hoesen and Pandya, 1975; Van Hoesen, 1982; Seltzer and Pandya, 1984; Suzuki and Amaral, 1984a) as well as in the superior temporal sulcus (Insausti et al., 1987; Good and Morrison, 1995) and the prefrontal cortex (Selemon and Goldman-Rakic, 1988). Many of these projections terminate contralaterally and ipsilaterally. The medial pulvinar, which receives projections from the IPL and projects to both the presubiculum and the EC, constitutes another major subcortical circuit.

There are at least two interpretations of this redundant multiplicity of interconnections. One possibility is that each projection may be specialized to convey highly specific information to the hippocampus. This was suggested by Goldman-Rakic et al. (1984) in their demonstration that prefrontal projections had two pathways, through the presubiculum and the parahippocampal cortex, into the hippocampal formation. Earlier studies of parietal-to-hippocampal connections similarly emphasized dual pathways through the presubiculum and the parahippocampal cortex (Seltzer and Van Hoesen, 1979; Seltzer and Pandya, 1984). Although it may still be convenient to consider the presubiculum and area TF as somehow “in parallel,” another, more likely possibility is that there is a more interactive, integrated architecture. The discovery that single IPL axons can branch to area TF, the perirhinal-entorhinal cortices, and the presubiculum would support this conclusion, as does the sheer density of the multiple pathways, which are well beyond two.

Convergence of parietal and temporal connections

In the context of visual perception, the relative separation of parietal and temporal processing streams (“dorsal” and “ventral” streams) has been a useful concept (Morel and Bullier, 1990). In contrast, the parietal and temporal subsystems dedicated to visuospatial and memory processing appear to share several points of convergence. Area 7a and area TF, in addition to their own reciprocal interconnections (Cavada and Goldman-Rakic, 1989; Andersen et al., 1990; Martin-Elkins and Horel, 1992), send partially convergent connections to CA1 (Rockland and Van Hoesen, 1999) and to the adjoining parts of the perirhinal and entorhinal cortices. Ventromedial area TE, which may be part of memory-related systems (Martin-Elkins and Horel, 1992), also projects to CA1 (Yukie and Iwai, 1988; Rockland and Van Hoesen, 1999; however, see also Suzuki and Amaral, 1990), to the border between the perirhinal and entorhinal cortices, and (seemingly unlike area TF) to the presubiculum as well. The convergence of projections from area 7a and ventromedial temporal areas at the border of perirhinal and entorhinal cortices may be significant for the marked impairment in visual recognition that results from ablations of the perirhinal region (Meunier et al., 1993; Suzuki et al., 1993; Buckley and Gaftan, 1998). It is worth noting, however, that rhinal lesions would also interrupt connections from the presubiculum to the rhinal cortex.

CONCLUSIONS

The entorhinal and perirhinal cortices have long been accorded privileged status in the communication between neocortical areas and the hippocampal formation, with particular emphasis on temporal cortical and parahippocampal pathways. Although, in fact, there is massive convergence of temporal cortical systems in the peri- and entorhinal cortices, what has become the standard view overlooks the fact that the presubiculum is also a component of the parahippocampal gyrus and itself is a major source of input to the EC. Presubicular input is dominated by the frontal and parietal lobes and the parts of the cingulate gyrus that are interconnected with them. Factoring in this presubicular input, the EC emerges all the more as a cortical area influenced by all lobes. Finally, the divergence of axon collaterals from the IPL adds a new level of integration to the corticohippocampal architecture and calls for careful reanalysis of the ventromedial temporal networks.

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LITERATURE CITED
