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MORPHOLOGICAL COMPARISON OF VISUAL PATHWAY PROJECTIONS TO
THE TEMPORAL LOBE FROM CORTICAL AREA VI AND THE TECTO-
RECIPIENT ZONE OF THE PULVINAR NUCLEUS IN THE TREE SHREW (TUPAIA
BELANGERI)

By

Donna Dillihay
B.S., B.A., University of Evansville, 1983

A Thesis
Submitted to the Faculty of
School of Medicine
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for the Degree of

Masters of Science

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MORPHOLOGICAL COMPARISON OF VISUAL PATHWAY PROJECTIONS TO
THE TEMPORAL LOBE FROM CORTICAL AREA V1 AND THE TECTO-
RECIPIENT ZONE OF THE PULVINAR NUCLEUS IN THE TREE SHREW (TUPAIA
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ABSTRACT

MORPHOLOGICAL COMPARISON OF VISUAL PATHWAY PROJECTIONS TO THE TEMPORAL LOBE FROM CORTICAL AREA V1 AND THE TECTO-RECIPIENT ZONE OF THE PULVINAR NUCLEUS IN THE TREE SHREW (*TUPAIA BELANGERI*)

Donna Dillihay

November 16, 2012

The secondary visual pathway, from the retina through the superior colliculus and pulvinar nucleus has been linked to spatial attention (Snow et al., 2009; Arend et al., 2008) movement planning and motor response to visual stimuli (Wilke et al. 2010; Grieve et al. 2000). The tree shrew temporal cortex receives input from the tecto-recipient pulvinar nucleus, composed of the dorsal (PD) and central (PC) subdivisions (Lyon et al. 2003a). We looked at the projections from this pathway to the temporal cortex and compared them to the V1 projections to the temporal cortex in the tree shrew. Our focus was to determine if there are differences in these projections that could further define their functional relationships. The characteristics we compared are the layers of termination, the axon and bouton density, the axon arbor shape and branching, axon caliber, bouton size, type and clustering.

In order to clearly identify the target temporal lobe areas of projections from V1 and the pulvinar, we mapped the architectonic features of the areas on a model brain using a computerized microscope system onto which we then mapped the V1 and

pulvinar nucleus projections. Our research found that the area to which the axons project defines the morphological characteristics of projections to that target area more than the source of the projection does. This is contrary to existing model of cortical areas receiving "driver" and "modulator" projections that have distinct morphological characteristics.

We found three projection "zones" within the TP and TD areas, TP representing the upper peripheral visual field, caudal TD representing the central visual field and rostral TD representing the lower peripheral visual field. Projections to each of these target zones had different morphological characteristics. This evidence would indicate that there are three functions represented in these cortical areas that combine the input of the primary and the secondary visual pathways.

The V1 and pulvinar nucleus tecto-recipient zone projections to the temporal cortex were thin, dense and moderately to heavily branched. The boutons were mostly small and numerous. V1 axons projecting to TP (V1-TP) and PD axons projecting to TP (PD-TP) have wide arbors of thin axons with extensive branching and many small boutons of mixed boutons en passant and terminal bouton type. V1 to TP axons are sparser than PD to TP axons and are more likely to give rise to boutons en passant. Pulvinar nucleus projections to caudal TD (PD-TD, PC-cTD) also result in wide arbors. These are dense and extensively branched with mixed thin and thick axons and many small to medium sized boutons. V1 projections to TD (V1-TD) and pulvinar nucleus projections to rostral TD (PC-rTD) are organized in narrow local arbors of mixed thin and thick caliber axons with sparse to moderate density and branching. Boutons are

small to medium sized. V1-TD axon branching is more sparse than PC-rTD axon branching. V1-TD axons have slightly more boutons than PC-rTD axons.

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CHAPTER 1: INTRODUCTION

In addition to the primary visual pathway from the retina, through the dorsal lateral geniculate nucleus (dLGN), to the striate cortex (V1), mammals have a secondary pathway from the retina through the superior colliculus and pulvinar nucleus to a variety of visual cortical areas including several in the temporal lobe. This secondary pathway has been linked to spatial attention (Snow et al., 2009; Arend et al., 2008), movement planning and motor response to visual stimuli (Wilke et al. 2010; Grieve et al. 2000). In studying this pathway, tree shrews are a good model animal because they are highly visual animals that exhibit the ability to move extremely quickly. They also have a large pulvinar nucleus and superior colliculus. Tree shrews are classified as the only member of order Scandentia but represent an early form of proto-primate (LeGros Clark, 1934; Lockett, 1980).

Earlier studies in tree shrews have defined temporal lobe areas that are part of the primary visual pathway, using connections to and from V1 and V2. First labeled in 1972 (Kaas et al., 1972), as area 19 (occipital cortex), the area of cortex along the ventrorostral border of V2 has since been identified as temporal cortex. Sesma et al. (1984) identified two areas labeled the temporal dorsal area (TD) and the temporal posterior area (TP). In 1994 Jain et al. reaffirmed the projections to TD and TP, and labeled a third area, the

temporal anterior area (TA). All three of these temporal cortex areas receive input from VI.

Further areas were located by Lyon et al. in 1998. The temporal inferior area is architectonically identified as an area of heavier myelination in the temporal lobe ventral to the TP-TD-TA band. Two other general areas were identified as the temporal anterior lateral area (TAL) and the temporal posterior inferior area (TPI). In 2009 Wong and Kaas architectonically defined additional temporal lobe areas using histochemistry (thionin processing for Nissl substance, Gallyas (1979) silver processing for myelin, cytochrome oxidase (CO; Wong-Riley, 1979) processing and intravenous injections of sodium sulfide with Ichinohe et al. (2004) protocol processing to visualize synaptic zinc) and immunocytochemistry (anti-parvalbumin antibody, anti-VGluT2 antibody and anti-SMI-32 antibody reactions). Their architectonic analysis replaced the TAL and TPI designations with the more clearly defined inferior temporal caudal area (ITc), inferior temporal intermediate area (ITi) and inferior temporal rostral area (ITr).

The tree shrew temporal cortex receives input from the tecto-recipient pulvinar nucleus, composed of the dorsal (PD) and central (PC) subdivisions (Lyon et al. 2003a). In 1988, Luppino et al. used retrograde tracing techniques to establish that TD and TP receive input from PD and PC, respectively. Lyon et al. (2003b) subsequently used retrograde tracing techniques to uncover pathways from PD to temporal cortical areas TP and TPI, from PC to temporal cortical area TD and from PP to temporal cortical areas TA, TI and TAL. Most recently, Chomsung et al. (2010) used anterograde tracing techniques to establish that projections from the PD and PC define two areas within the

temporal cortex ventrorostral to the V2 border, one caudal and one rostral. That study did not differentiate the architectonic temporal lobe areas along that border.

Because the primary and secondary pathways converge in the temporal cortex, it remains unclear whether the response properties of temporal cortex neurons are defined primarily by corticocortical or thalamocortical pathways. By comparing the laminar terminations of corticocortical axons Fellman and Van Essen (1991) proposed a hierarchical arrangement of visual cortical pathways. In essence, it was proposed that corticocortical projections that terminate primarily in layer 4 are “feedforward” or driver projections, while corticocortical projections that terminate primarily above or below layer 4 are “feedback” or modulatory inputs. Using this scheme, many areas in the temporal cortex receive feedforward, driver inputs from V1. However, projections from the tecto-recipient zones of the pulvinar to the temporal lobe have been found to terminate primarily in layer 4 as well (Chomsung et al. 2010).

In this study we examined the primary pathway connections from V1 to the temporal lobe and compared them to the secondary pathway connections to the temporal lobe from the tecto-recipient zones of the pulvinar nucleus (PD and PC). Our focus was to determine if there are differences in these projections that could further define their functional relationships. The characteristics we compared are the layers of termination, the axon and bouton density, the axon arbor shape and branching, axon caliber, bouton size, type and clustering. In order to clearly identify the target temporal lobe areas of projections from V1 and the pulvinar, we mapped the architectonic features of the areas on a model brain using a computerized microscope system onto which we then mapped the V1 and pulvinar nucleus projections.

CHAPTER 2:

METHODS

A total of 12 adult (average weight 172 g) tree shrews (*Tupaia belangeri*); 5 males and 7 females, were used for these experiments. Fifty micron thick coronal brain sections every 300 microns from 4 tree shrews were stained for Wisteria Fluoribunda Agglutinin (WFA) (2 tree shrews) or Parvalbumin (2 tree shrews) and used to identify, calculate the size of and map the locations of the temporal lobe divisions. These divisions were identified by the naming conventions established by Wong and Kaas (2009) as the temporal anterior area (TA), temporal dorsal area (TD), temporal posterior area (TP), temporal inferior area (TI), inferior temporal caudal area (ITc), inferior temporal intermediate area (ITi) and inferior temporal rostral area (ITr; Fig. 5). Five tree shrews were used to trace projections to the temporal lobe labeled by injections of the anterograde tracer biotinylated dextran amine (BDA) (5 tree shrews) and cell bodies in the temporal lobe labeled by injections of the retrograde tracer Cholera Toxin B Subunit (CTB) (1 tree shrew) in V1. Seven tree shrews were used to investigate the distribution and morphology of the projections to the temporal lobe labeled by injections of BDA in two areas of the pulvinar, the dorsal pulvinar (PD) (3 tree shrews) and the central pulvinar (PC) (4 tree shrews). Several of these animals were also used for previous studies (Chomsung et al. 2008, 2010). All methods were approved by the University of

Louisville Animal Care and Use Committee and conform to the National Institutes of Health guidelines.

Tracer Injections

Tree shrews that received BDA (3000 MW; Molecular Probes, Eugene, OR) and CTB (List Biological Laboratories, Inc., Campbell, CA; catalogue #105) injections were initially anesthetized with intramuscular injections of ketamine (100 mg/kg) and xylazine (6.7 mg/kg). Additional supplements of ketamine and xylazine were administered approximately every 45 min to maintain deep anesthesia through completion of the tracer injections. The heart rate was continuously monitored with a MouseOx pulse oximeter (STARR Life Sciences Corp., Pittsburgh, PA). Prior to injection, the tree shrews were placed in a stereotaxic apparatus and prepared for sterile surgery. A small area of the skull overlying the dorsal pulvinar nucleus, central pulvinar nucleus or V1 was removed and the dura reflected. For all of the pulvinar injections, and 1 of the V1 injections, a glass pipette containing BDA (5% in saline, tip diameter 2 μm) or BDA + CTB (5% BDA + 1% desalted CTB in 0.1 M phosphate buffer, pH 6.0; tip diameter 2 μm) was lowered vertically and the tracer was ejected iontophoretically (2 μA positive current for 15--30 min). For the remaining injections, a 1- μL Hamilton syringe containing 0.1--0.3 μL of BDA + CTB (5% BDA + 1% desalted CTB in 0.1 M phosphate buffer, pH 6.0) was lowered vertically into the visual cortex and the tracers were ejected via pressure. After a 7-day survival period, the tree shrews were given an overdose of sodium pentobarbital (250 mg/kg) and were perfused through the heart with Tyrode solution, followed by a fixative solution of 2% paraformaldehyde and 2% glutaraldehyde or 4% paraformaldehyde in 0.1 M phosphate buffer, pH 7.4 (PB).

Histochemistry to reveal tracers

Tree shrew brains were removed from the skull and cut on the coronal plane using stereotaxic blocking. They were sectioned into 50- μ m-thick sections using a vibratome (Leica VT100E, Leica Microsystems, Bannockburn, IL) and collected in a solution of 0.1 M PB. In some cases, sections were preincubated in 10% methanol in PB with 3% hydrogen peroxide (to react with the endogenous peroxidase activity of red blood cells not removed during the perfusion).

The BDA was revealed by incubating sections in a 1:100 dilution of avidin and biotinylated horseradish peroxidase (ABC; Vector Laboratories, Burlingame, CA, USA) in phosphate-buffered saline (0.01 M PB with 0.9% NaCl, pH 7.4; PBS) overnight at 4°C. The sections were subsequently rinsed three times in PB (10 min each), reacted with nickel-intensified 3,3'-diaminobenzidine (DAB) for 5 min, and washed in PB. DAB-labeled sections were mounted on slides for lightmicroscopic examination.

Immunocytochemistry to reveal architectonic structure

Sections from previously tracer injected, perfused tree shrew brains which were sectioned into 50- μ m-thick sections were used for immunocytochemistry. To reveal the architectonic structure of the different temporal lobe areas in the tree shrew cortex, biotinylated-WFA (Vector Laboratories, Burlingame, CA, diluted 1:100), an antibody against parvalbumin (Sigma, St. Louis, MO; diluted 1:5000), or myelin staining (2% OsO₄ in 0.1 M PO₄ buffer for 1 hour) were employed. To reveal the distribution of parvalbumin, selected sections were incubated overnight in the primary antibody. The next day sections were rinsed in 0.1 M phosphate buffer, incubated 1 h in biotinylated goat-anti-mouse (1:100), rinsed again and incubated for 1 h in avidin and biotinylated-

horseradish peroxidase (ABC solution) at a dilution of 1:100, before reacting with nickel-enhanced diaminobenzidine (DAB). To reveal the distribution of WFA binding, selected sections were incubated overnight in the WFA. The next day sections were rinsed in 0.1 M phosphate buffer, and incubated for 1 h in ABC solution before reacting with nickel-enhanced DAB.

Light Microscopy and Computer Generated Figures

A Neurolucida system (MicroBrightField, Inc., Williston, VT) and light microscope (Nikon Eclipse E800; Nikon Corporation, Tokyo, Japan) were used to generate micrographs of projections revealed by tracer injections and architectonic features revealed with staining. Using Photoshop software (Adobe Systems, Inc., San Jose, CA) and Roxio Photosuite software (Corel Corporation, Ottawa, Ontario, Canada), the brightness, contrast and cropping were adjusted to optimize the images.

3-D Anatomical Reconstruction

Using fifty micron sections, every 3rd section was used for staining with WFA or Parvalbumin to reveal variations in structure throughout the cortex. The sections were compared in detail to the Stereotaxic Brain Atlas of the Tree Shrew (*Tupaia Glis*) (Tigges and Shantha 1969) and the AP coordinate for each section was identified. If the stereotaxic blocking resulted in slanting of the sections as compared to the atlas, the AP coordinates of the top and the bottom of the section were identified so that variations in cortical area size could be adjusted to the standard (Atlas). Brain size measurements for each tree shrew brain were taken to standardize measurements to the atlas, which uses an average of 14 brains with a weight averaging 3.2 grams. The maximum dorsal-ventral measurement of the cortex as shown in the atlas is 13mm. Final cortical measurements

for each temporal lobe area are reported both as values in relation to the standard (Atlas) and as ratios based on cortical dorsal-ventral measurement, as would be used in experimental injections and surgeries.

The left-hand side of each section of a selected model brain was traced and key markers drawn using Neurolucida system (MicroBrightField, Inc., Williston, VT). Sections were aligned using center points at the top and bottom of each section and the ventral, lateral and medial surfaces of the left-hand cortex. The drawings were used to produce a 3d reconstruction of the tree shrew occipital and temporal cortical areas with the Neurolucida 3d functionality. Next the areas of projection from V1 to the temporal cortex were outlined on each section of tree shrew brain prepared as discussed above. These projections were overlaid on the temporal lobe areas and a 3d reconstruction produced. Sections from a tree shrew brain with a single large injection combining CTB and BDA was used to plot the distribution of cells with retrograde tracer from V1 to the temporal lobe. Finally, the areas of projection from the pulvinar dorsal (PD) and central (PC) areas, produced in a previous project by this lab (Chomsung et al. 2010), were overlaid on the drawings and a 3d reconstruction produced.

Bouton Size and Quantity

Microphotographs of the axons and boutons for each projection type were analyzed using ImageJ. A sample range of boutons were measured categorized as small (< 1 μm , average 0.8 μm), medium (1-2 μm , average 1.5 μm) or large (>2 μm , average 2.2 μm). Boutons were then counted by category in the microphotographs for each projection. Statistics such as total area analyzed, boutons per area and percent large, medium and small were calculated.

CHAPTER 3:

RESULTS

Temporal Cortical Area Definitions

In order to clearly identify the target temporal lobe areas of projections from V1 and the pulvinar tecto-recipient zones, we architectonically mapped each area on a model tree shrew brain. In earlier studies of the tree shrew, cortical architectonics definitions of the temporal lobe areas have been depicted primarily on a flat cortex. In our study we used coronal sections and identified the delineations in 50 μm thick sections separated by 300 μms . Three stains were used, as described in the methods section, to identify area demarcations: wisteria floribunda lectin (WFA), a parvalbumin antibody (Parv) and osmium (to stain myelin). We referenced the architectonics of Wong and Kaas (2009) to name the areas consistently with that work. We used the Stereotaxic Brain Atlas of the Tree Shrew (*Tupaia Glis*) (Tigges and Shantha 1969) to identify the location of each section and standardized measurements across animals to the atlas. This provided us with a framework within which to further characterize the V1, PD and PC projections and their targets.

The areas that concern this present study are those that were previously labeled by Wong and Kaas (2009) as TP, TD, TA and TI. These are the areas that we found were targets of V1 and/or pulvinar connections. The remaining temporal lobe areas are

covered for completeness of the coronal architectonic map. We have also commented briefly on V2, since there are heavy V1 connections to that area that have been studied and defined previously, and are useful as comparisons in defining the other connections.

Architectonic characteristics of the different areas of the tree shrew temporal lobe (and V2) revealed with WFA and Parv staining are summarized in tables 1, 2 and 3, and are discussed below. Table 4 summarizes the locations and sizes of the temporal lobe areas identified.

Secondary Visual Area (V2)

Area V2 is apparent in both WFA and Parvalbumin staining as a 1.6 mm wide area stretching the length of the ventrorostral border of V1 as previously identified with other staining (Sesma et al. 1984; Cusick et al. 1985).

As illustrated in figure 1A, with WFA staining, V2 has a dark layer 4 and a light layer 5a, while V1 has a light layer 4 and darker layer 5. V2 layers 3b and 5b also appear dark. V2 layer 6 is slightly lighter than in V1. The layer 4 transition from light to dark is the best marker for the boundary between V1 and V2 using WFA staining.

With Parvalbumin staining (Fig. 1D), V1 exhibits dark layers 4 and 6. In contrast, V2 has a only a faint darkening of neuropil in layer 4, while the darker color of layer 6 is not due to neuropil staining, but rather to denser groupings of cell bodies. Layer 5 neuropil is lightly stained in both V1 and V2. In layer 2 of V2 bands of cells are visibly stained (Fig. 1G). Layers 3-4 have scattered stained cells. Layer 5 has very few stained cells.

In coronal sections, V2 reaches the whole length of V1's border (from about P2.5 to about A7.0). It appears widened to 1.9 mm in coronal sections at the far caudal end due to the cutting angle (Fig 2 and Table 4).

Temporal Posterior Area (TP)

While WFA in V2 creates a wide dark area incorporating layers 3b and 4, TP (Fig. 1B) has a broad homogenous medium coloring in layers 1-3, followed by thin, half-layer alternating bands of light and dark in layers 4-6. Layer 4a is a dark band, followed by layer 4b as a light band, then 5a as a dark band, 5b as a light band and 6 as a dark band.

Parv stained sections (Fig. 1E) show TP to have a darker band of neuropil staining in layer 4 compared to V2. Like V2, a band of stained cells can be seen in layer 2 and in layer 6 (Fig. 1H). There are scattered stained cells throughout layers 3 and 4, and sparse cells in layer 5.

TP is the first temporal lobe area to appear caudally in coronal slicing (Fig 2). TP is fairly large area located at the caudal end of the ventrorostral border of V2. TP is approximately 3 mm in length along V2 border (P2.0 to A0.5) (Fig. 2 and Table 4). This area varies in size along the dorsoventral dimension as we move along the caudal-rostral dimension. Its maximum dorsoventral length is 5.2 mm. We measured the area of TP at the cortex surface in our reconstruction (Fig. 5) as approximately 8.6 mm², which is smaller than the estimate by Sesma et al. (1984) of 10mm². Our 3-D reconstruction shows that the area tapers dramatically at the ventral end. This makes the overall area smaller than would be calculated if the area were more rectangular.

Table 1: WFA Staining Results

Lay -er	V2	TP	TD	TA	TI	ITc	ITi	ITr
1	medium	medium	medium	medium	medium	medium	medium	dark
2	medium	medium	medium	medium	medium	medium	medium	dark
3	a medium b dark	medium	dark	dark	dark	patchy	medium	dark
4	dark	a dark b light	light	dark	dark	patchy	medium	dark
5	a light b dark	a dark b light	a dark b v.light	a dark b light	a dark b light	a dark b light	patchy	dark
6	medium	dark	medium	medium	medium	dark	patchy	patchy

Table 2: Parvalbumin Neuropil Staining Results

Layer	V2	TP	TD	TA	TI	ITc*	ITi	ITr
1	medium	light	dark	dark	dark	dark	dark	Dark
2	medium	medium	light	dark	patchy	light	light	faint
3	medium	medium	light	dark	patchy	light	light	faint
4	medium	dark	dark	dark	dark	dark	thin, medium	light
5	faint	light	light	faint	light	light	light	faint
6	medium	medium	dark	dark	dark	dark	thin, medium	light

*overall darker than TD

Table 3: Parvalbumin Cell Staining Results

Layer	V2	TP	TD	TA	TI	ITc	ITi	ITr
2	band	band	band	band	scattered	band	band	band
3	scattered	scattered	scattered	few	scattered	scattered	scattered	scattered
4	scattered	scattered	scattered	few	scattered	scattered	band	scattered
5	few	few	scattered	very few	scattered	scattered	few	very few
6	band	band	scattered	few	scattered	scattered	band	scattered

Table 4: Summary of Temporal Lobe Area locations and sizes in Stereotaxic Measurements

Region	Area (mm ²)	Caudal End		Rostral End	
		AP	DV	AP	DV
TP	8.6	P2.0	5.0 - 5.5	A0.5	3.0 - 3.7
TD	20.5	A0.5	2.3 - 3.1	A6.0	1.1 - 1.8
TA	7.0	A3.8	0.6 - 1.0	A6.8	0.5 - 0.8
TI	2.7	A4.7	3.4 - 4.6	A6.2	2.9 - 3.2
ITc	7.7	A0.5	5.7 - 7.0	A4.6	3.4 - 4.6
ITi	9.0	P0.1	7.1 - 8.6	A5.6	3.9 - 4.9
ITr	4.7	A1.7	8.7 - 9.3	A5.6	5.0 - 6.0

Figure 1: WFA and Parv Staining of V1, V2, TP and TD

WFA staining (A-C) and Parv staining (D-I) identifying the border between V1 and V2 (A,D), V2 and TP (B, E) and V2 and TD (C,F). Magnification of parv staining to view neuropil and cell bodies (G-I). Arrows are dense cell bands. Scale bar in F covers A-F. Scale bar in I covers G-I.

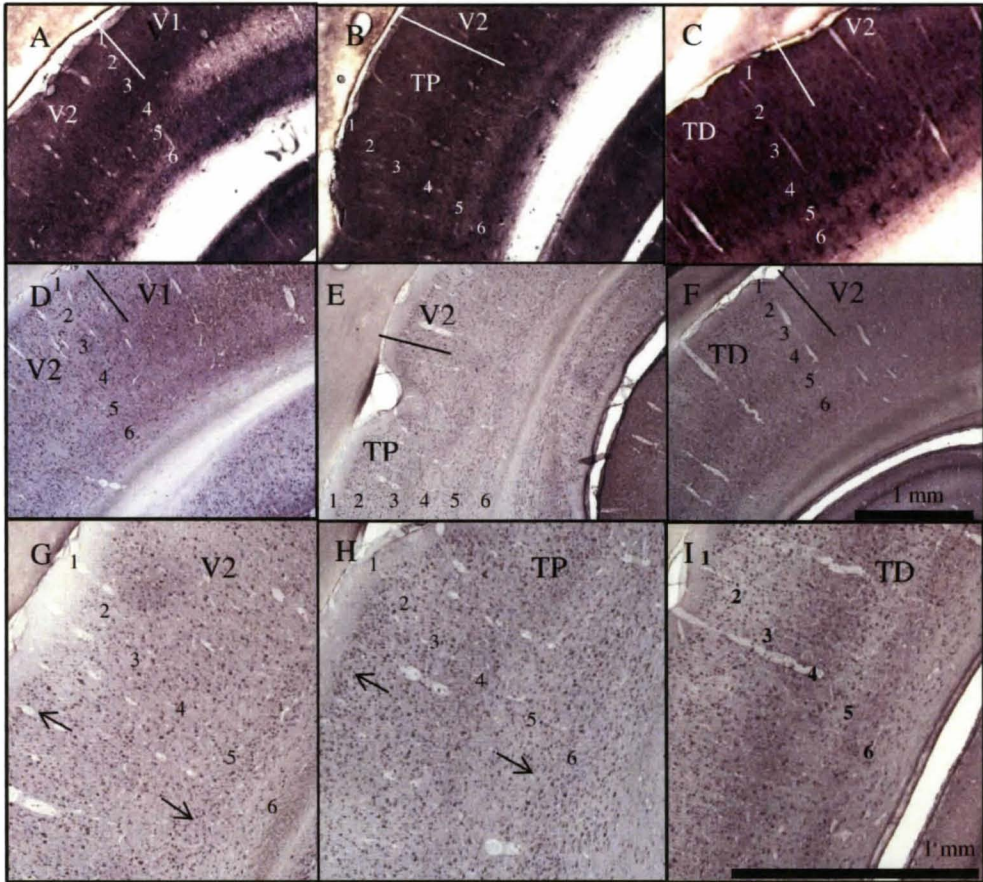
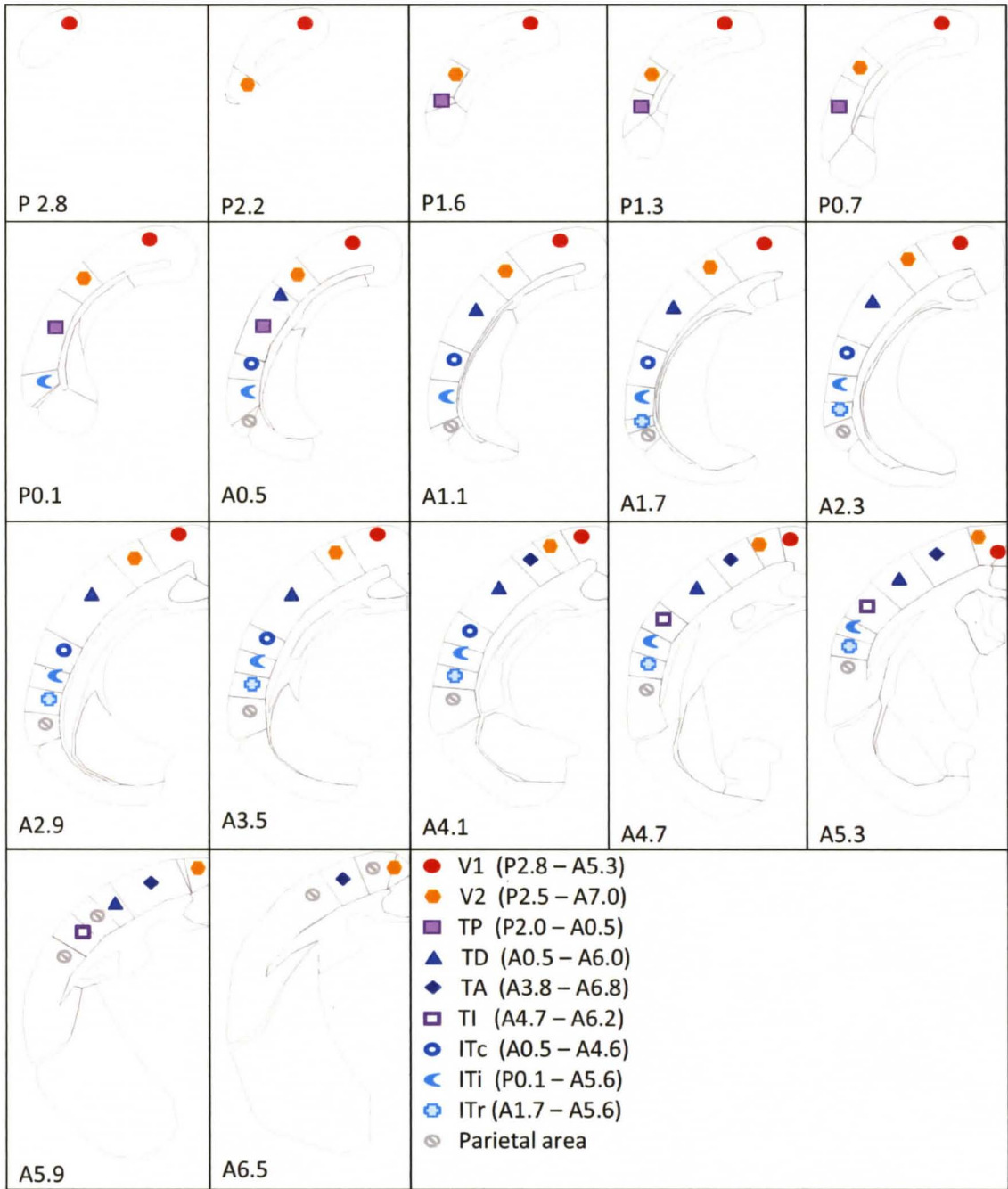


Figure 2: Coronal sections of the tree shrew cortex

Coronal sections of the tree shrew cortex with occipital and temporal areas marked. Sections are arranged by stereotaxic AP coordinates.



Temporal Dorsal Area (TD)

With WFA staining (Fig. 1C), TD layer 3 can be seen as a wide dark band followed by a light layer 4 band. This stands out against V2 which has a dark layer 4 with a dark 3b. In TD, Layer 5a is dark and 5b is very light. Layer 6 is medium in color. Against V2, the stripping created by the light and dark layers is clearly visible.

With Parv staining (Fig. 1F), TD has significantly darker and thicker layers 4 and 6, and lighter layers 2, 3 and 5 than V2. The neuropil in layers 1,4 and 6 stain darkly, and in layers 2, 3 and 5 stain lightly. There are uniformly scattered stained cells throughout layers 2-6 (Fig. 1I). Parv staining clearly delineates the TD area. When looking at the border between TP and TD, TP is distinguished from TD by being darker in layers 2-4 and lighter in layer 5. TP layer 4 is also a tighter band than seen in TD.

TD extends along the ventrorostral border of V2, next to TP, for about 5.5 mm (A0.5 to A6.0) (Fig. 2 and Table 4). Its caudodorsal-to-ventrorostral dimension, moving away from the V2 border, is wide in the middle, narrowing on both ends, being 3.4 mm at its widest. In Sesma et al. (1984), the TD area was identified as being 5-6 mm along the V2 border and 2-3 mm wide moving away from the V2 border. Our values are within a range agreeing generally with these findings, but being slightly larger.

The area of TD in our model was calculated as 20.5 mm², larger than the estimate of 13 mm² by Sesma et al. (1984). The maximum width of the TD area dorsoventrally on a coronal section is 5.6 mm due to the approximately 45' angle of the V2 border.

Temporal Anterior Area (TA)

TA is not distinguishable from V2 with WFA staining (Fig. 3A). TA stains darkly in layers 3 and 4. Layer 5a is darkly stained while layer 5b is lightly stained. It is also difficult to see the difference between TA and TD, which is only that layer 4 is light in TD and dark in TA.

Parv stains TA (Fig. 3D) neuropil more darkly in layers 1-4 and 6 than it stains V2. Layers 2-3 are darker than in TD. Layer 5 neuropil stains only faintly, as it does in V2 and TD. There were few scattered stained cells in layers 2- 4 and 6 (Fig. 3F), even fewer stained cells in layer 5, and a band of stained cells at the top of layer 2. The bands in layers 2-4 in TD stand out against the lack of banding in TA.

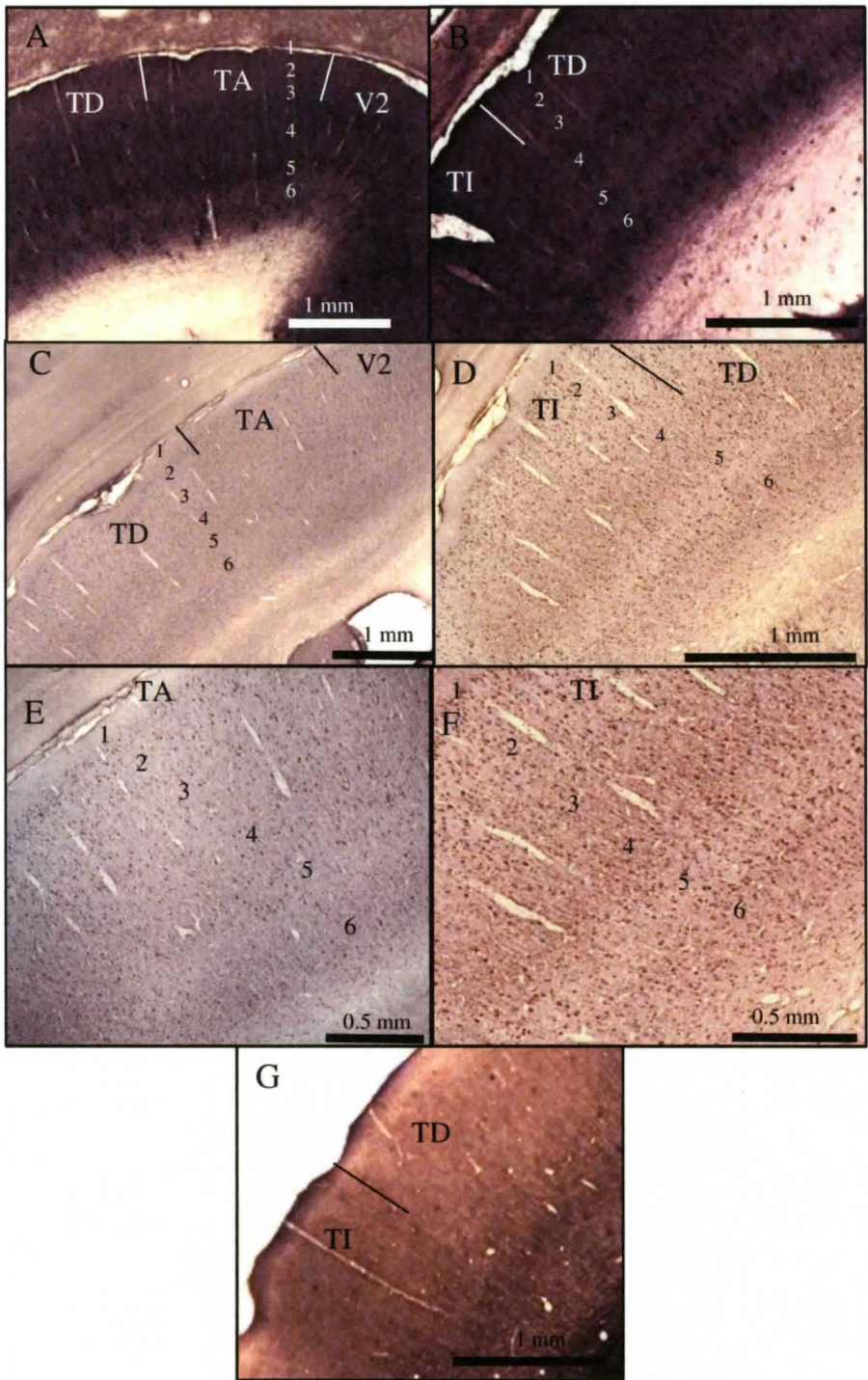
The temporal anterior area (TA) follows TD on the ventrorostral V2 border and extends about 3.0 mm along that border (A3.8 to A6.8) (Fig. 2 and Table 4). The maximum width moving away from the V2 border ventrorostrally is about 1.5 mm. We measured the area of TD in our model to be approximately 7.0mm².

Temporal Inferior Area (TI)

With WFA staining (Fig. 3B), TI looks very similar to TD. The area overall is slightly darker, but the distinction between layers is the same. With Parv staining (Fig 2E,G), TI has a much darker staining of layer 4 neuropil than TD and a lower population of stained cell bodies in layer 4. Neither stain was a good method of identifying TI. However, TI is much more heavily myelinated than surrounding areas (Wong and Kaas, 2009), so we used myelin staining to verify the location of TI (Fig 2C).

Figure 3: WFA and Parv Staining of V2, TA, TD and TI

WFA staining (A-B), Parv staining (C-F) and myelin staining (G) identifying the border between V2, TA and TD (A,C); TD and TI (B,D,G). Magnification of parv staining to view neuropil and cell bodies (E,F). Scale bars as shown.



The temporal inferior area (TI) is located ventrostrally to TD. Unlike the findings in Wong, Kaas 2009, we found with both staining and projection mapping that TI borders TD. The border between the areas is about 1.4 mm long (A4.7 to A6.2) (Fig. 2 and Table 4). The maximum caudodorsal to ventrostral width of TI is about 1 mm. The area of TI is approximately 2.7 mm².

Inferior Temporal Caudal Area (ITc)

When stained with WFA, ITc has a patchy appearance in layers 3-4 that is distinct from staining in previous areas (Fig. 4A). Like other areas reviewed, layers 5a and 6 stain darkly while 5b is light. With Parv staining, ITc is very similar to TD (Fig. 4D). The neuropil is overall darker, but it has the same characteristics of a dark band of neuropil in layers 1, 4 and 6, light staining in layers 2,3 and 5, and uniformly scattered stained cells through layers 2-6 with a band of cells in layer 2. It is possible to distinguish the TD to ITc border by the change to a darker overall area.

ITc begins rostral to TP and ventral to TD. It extends along the ventrostral border of TD about 4.8 mm (A0.5 to A4.6). At its widest moving away from the TD border in a caudodorsal-to-ventrostral direction it is about 2.0 mm wide. Its area is approximately 7.7 mm².

Inferior Temporal Intermediate Area (ITi)

With WFA staining, ITi exhibits patchiness in layers 5 and 6 without any other layering characteristics (Fig. 4B,C). This makes ITi fairly distinct, appearing next to ITc as an un-striped area with patchiness at the bottom.

Parv staining in ITi (Fig. 4E,F) produces thin medium stained bands of neuropil in layers 4 and 6, and dark staining of layer 1. The banding in ITi is much lighter and thinner than in ITc. There are bands of heavier cell staining in layers 2, 4 and 6.

ITi runs along the ventral border of TP and then ITc (P0.1 to A5.6). Its length on the caudoventral to rostradorsal diagonal is about 6.8 mm. It is widest at the caudoventral end, measuring about 2.0 mm moving ventrorostrally from the TP border. The rostradorsal two thirds of ITi is about half that width. The area of ITi is about 9.0 mm².

Inferior Temporal Rostral Area (ITr)

WFA staining of ITr stains a darker shade overall than ITi, and is patchy only in layer 6 (Fig.4C). Other than the patches, ITr has no distinguishing features.

With Parv staining, ITr stains overall very lightly, displaying faint bands of darker neuropil in layers 4 and 6 and dark neuropil in layer 1 (Fig. 4F). Layer 2 has a band of stained cells. There are scattered stained cells throughout the rest of ITr with a noticeably lower number in layer 5.

ITr runs along the ventrorostral border of ITi (A1.7 to A5.6). The width moving away from the ITi border ventrorostrally is about 1 mm. The area of ITr was measured as 4.7 mm² in our model.

Figure 4: WFA and Parv Staining of TD, ITc, ITi and ITr

WFA staining (A-C) and Parv staining (D-F) identifying the borders between TD and ITc (A,D), ITc and ITi (B, E) and ITi and ITr (C,F). Scale bar in F covers A-F.

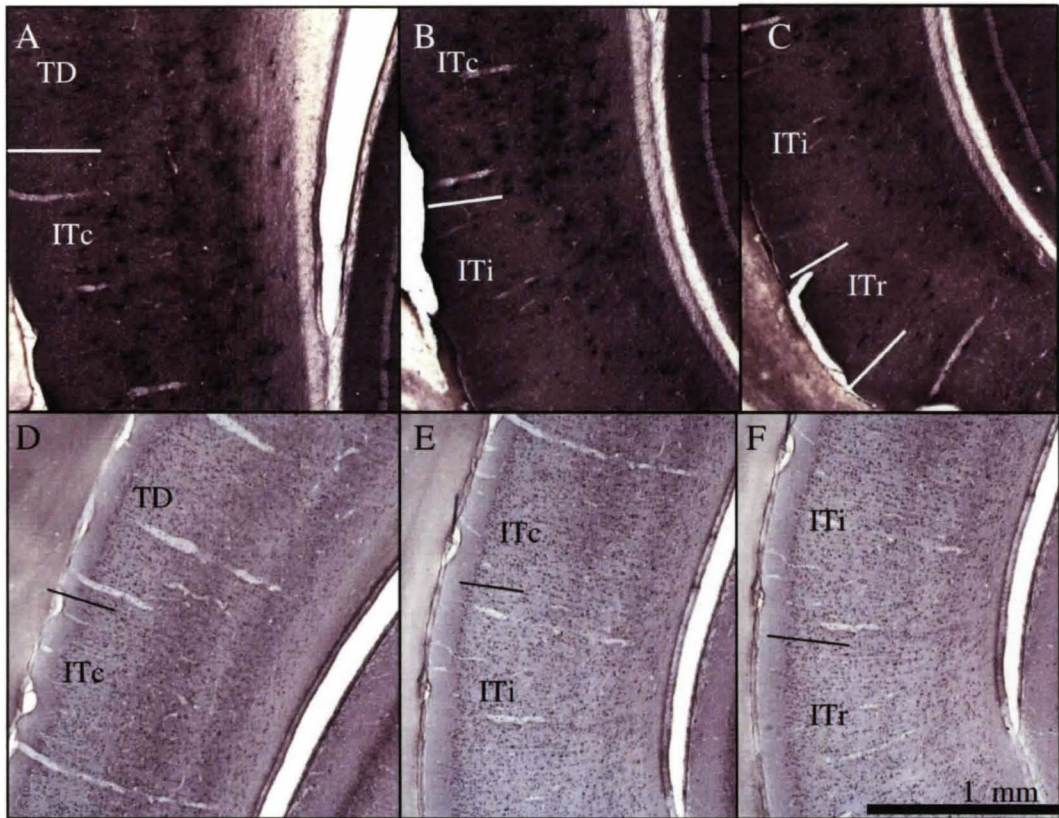
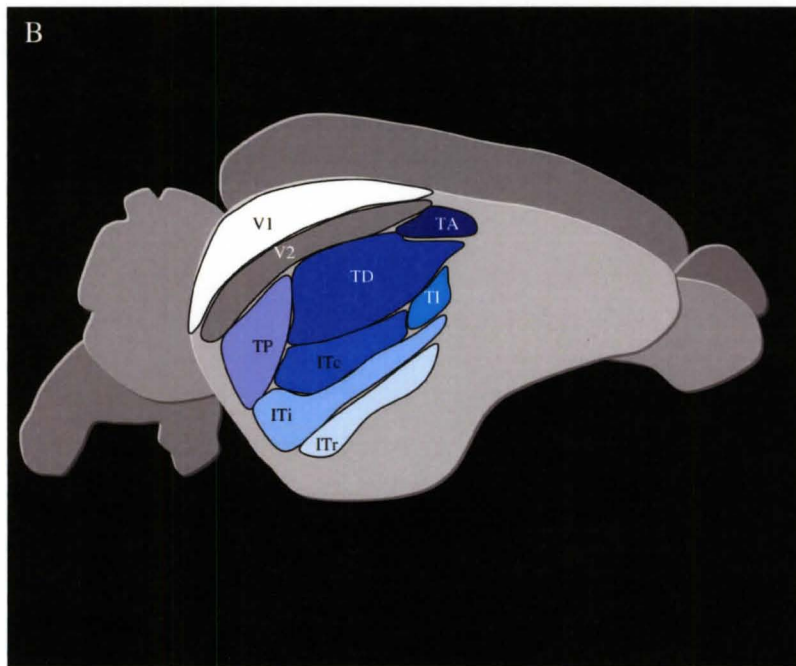
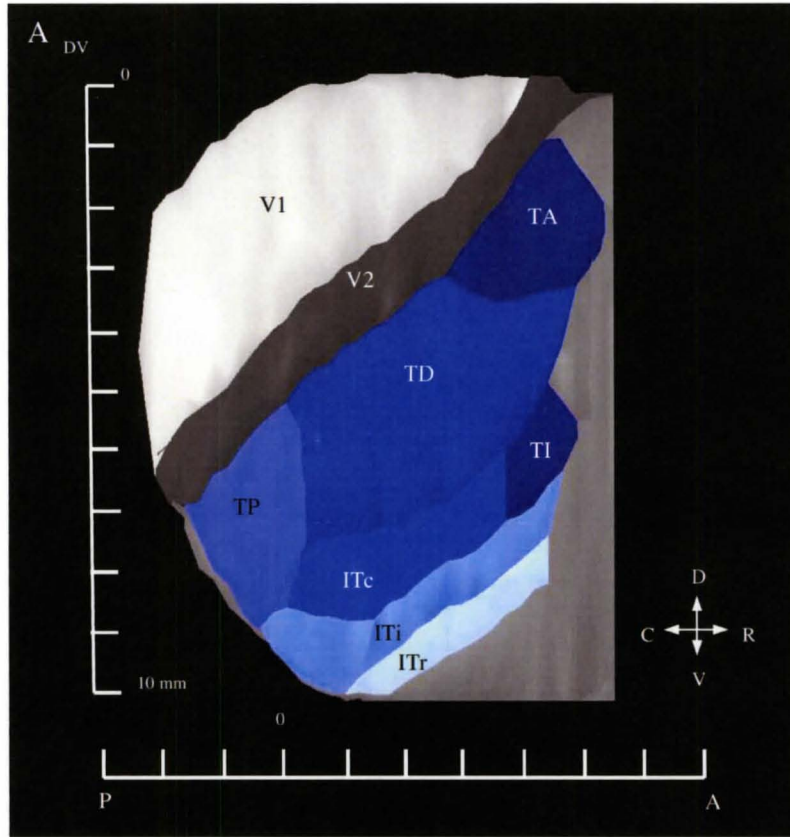


Figure 5: 3D reconstruction of the tree shrew occipital and temporal lobe areas

3D reconstruction of the tree shrew occipital and temporal lobe areas. A: Reconstructed areas with stereotaxic measurement scales. B: Reconstructed areas set in the outline of a tree shrew brain.



Projections to Temporal Cortical Areas

The characteristics of axons and boutons by projection source and target are summarized in tables 5-8 and are discussed below.

Primary Visual Area (V1) Projections

Five injections in the primary visual area V1 were used to trace connections from that area to various locations in the tree shrew temporal lobe. One injection was placed centrally and allowed to expand to a large area of V1 through extended injection time. Four other injections placed in locations around and sometimes overlapping that area were evaluated to determine what affect the position of the injection had on the projections to the temporal lobe. These injection sites are illustrated in Figure 6.

Secondary Visual Area (V2)

V1 projects to the entire extent of V2. Previous studies in various animal models have shown that V1 projections to V2 are topographic (Sesma et al. 1984;Kaas et al. 1989;Pospichal et al. 1996). Projections from V1 to V2 (V1-V2 axons) are distinct but interconnecting patches of long axons that form tight vertical columns (Fig. 7A,B). These patches are about 0.2mm wide and display a large number of densely distributed collaterals as the axons travel from layer 6 to layer 1 (Fig. 7C).

The densest area of V1-V2 axon branching and termination is in layer 4. The axons are thin with many small-to-medium sized boutons and some large boutons. These are primarily boutons en passant ("beads-on-a-string") with a few terminal boutons seen (Fig. 7D).

Temporal Posterior Area (TP)

Of the five V1 injections mapped in *Neurolucida*, three projected to TP (Fig 9A,B). As mentioned in Lyon et al. 1998 with regard to injections in V2 projecting to TP, we found similarly that our most rostral injection in V1 did not project to TP. An additional finding in our study was that our far medial injection also did not project to TP, nor did it appear that a large portion of our central injection projected to TP. The area of injections that projected to TP is the peripheral upper vision field (Fig. 9B).

Corticocortical projections to area TP that originate from V1 (V1-TP axons; Fig. 8A,E) differ substantially from V1-V2 axons. Whereas the V1-V2 projections form patches approximately 0.2 mm wide, the V1-TP patches are approximately 0.6 mm wide. The axons are short and bushy, spreading horizontally rather than vertically. Arbors are sparse. The densest axons and terminals are found in layer 4, with some projecting into layer 3. We did not find any V1-TP axons in layer 1. There are more terminals on V1-TP axons than there were in the V1-V2 axons, but there are far fewer axons, resulting in overall far fewer boutons. While there are a few larger caliber V1-TP axons in the layer 4 patches, the caliber of the axons overall is fine. The V1-TP terminals vary in size but the majority are small. We observed none of the largest boutons that could be found in V1-V2 projections. The V1-TP boutons are an even mix of boutons en passant and terminal boutons, where V1-V2 projections had many more boutons en passant.

Table 5: Bouton measurements and counts by axon projection source and target

	Avg Size (μm^2)	Total # per μm^2	Large (avg 2.2 μm^2)		Medium (avg 1.5 μm^2)		Small (avg 0.8 μm^2)	
			# per μm^2	%	# per μm^2	%	# per μm^2	%
V1-V2	1.15	18.57	2.34	12.59	4.68	25.19	11.55	62.22
V1-TP	0.89	6.70	0.09	1.40	0.65	9.77	5.96	88.84
PD-TP	0.85	19.40	0.22	1.13	0.87	4.50	18.30	94.37
V1-TD	0.99	18.18	0.94	5.15	3.18	17.50	14.06	77.36
PD-TD	1.06	47.43	3.15	6.64	11.04	23.27	33.24	70.09
PC-cTD	0.93	16.71	0.44	2.61	2.18	13.06	14.09	84.33
PC-rTD	1.06	11.01	0.59	5.38	2.96	26.91	7.45	67.71
V1-TA	1.21	5.89	0.98	16.67	1.45	24.60	3.46	58.73
PC-TA	1.71	1.26	0.61	48.15	0.42	33.33	0.23	18.52
PP-TA	1.95	1.03	0.80	77.27	0.09	9.09	0.14	13.64
V1-TI	0.88	2.15	0.00	0.00	0.23	10.87	1.92	89.13
PP-TI	1.42	2.48	0.56	22.64	1.08	43.40	0.84	33.96

Table 6: Axon and bouton volume by axon projection source and target

	Layer 1 axons and terminals	Layer 2 axons and terminals	Layer 3 axons and terminals	Layer 4 axons and terminals	Layer 5 axons and terminals	Layer 6 axons and terminals
VI-V2	few	few	many	majority	few	few
VI-TP	none	none	few	majority	none	none
PD-TP	few	few	few	majority	none	none
VI-TD	few	few	few	majority	few	few
PD-TD	few	few	many	majority	none	none
PC-cTD	none	none	few	majority	none	none
PC-rTD	few	few	many	majority	none	none
VI-TA	none	none	many	majority	none	none
PC-TA	none	none	many	majority	majority	none
PP-TA	none	none	many	majority	majority	none
VI-TI	none	none	majority	majority	none	none
PP-TI	few	few	majority	majority	none	none

Table 7: Axon characteristics by axon projection source and target

	Arbor localization	Arbor density	Axon branching	Axon thickness
V1-V2	local	moderate	moderate	thin
V1-TP	wide	sparse	extensive	thin
PD-TP	wide	dense	extensive	thin
V1-TD	local	moderate	sparse	mixed
PD-TD	wide	dense	extensive	mixed
PC-cTD	wide	dense	extensive	mixed
PC-rTD	local	moderate	moderate	mixed
V1-TA	wide	moderate	extensive	mixed
PC-TA	wide	sparse	moderate	thick
PP-TA	wide	sparse	sparse	thick
V1-TI	local	sparse	sparse	thick
PP-TI	local	sparse	sparse	thin

Table 8: Bouton characteristics by axon projection source and target

	Bouton Size	Bouton quantity	Boutons en passant	Terminal boutons	Bouton grouping
VI-V2	medium	many	many	sparse	scattered
VI-TP	small	moderate	moderate	moderate	scattered
PD-TP	small	many	sparse	moderate	clustered
VI-TD	small-medium	many	many	sparse	scattered
PD-TD	small-medium	very many	many	sparse	scattered
PC-cTD	small-medium	many	moderate	moderate	scattered
PC-rTD	small-medium	moderate	many	moderate	scattered
VI-TA	medium-large	sparse	many	moderate	scattered
PC-TA	medium-large	sparse	many	sparse to none	scattered
PP-TA	medium-large	sparse	many	sparse to none	scattered
VI-TI	small	sparse	sparse	many	scattered
PP-TI	medium-large	sparse	many	sparse	scattered

Figure 6: V1 Injection Sites

Injection 1 at P0.0, L2.0 (case 11-06A); Injection 2 at P0.5, L1.0 (case 8-06-2D right); Injection 3 at A1.5, L0.5 (case 3-04-1AB left); Injection 4 at P1.0 L1.0 (case 8-06-2D left); Injection 5 at P0.5, L3.0(case 3-04-1AB right)

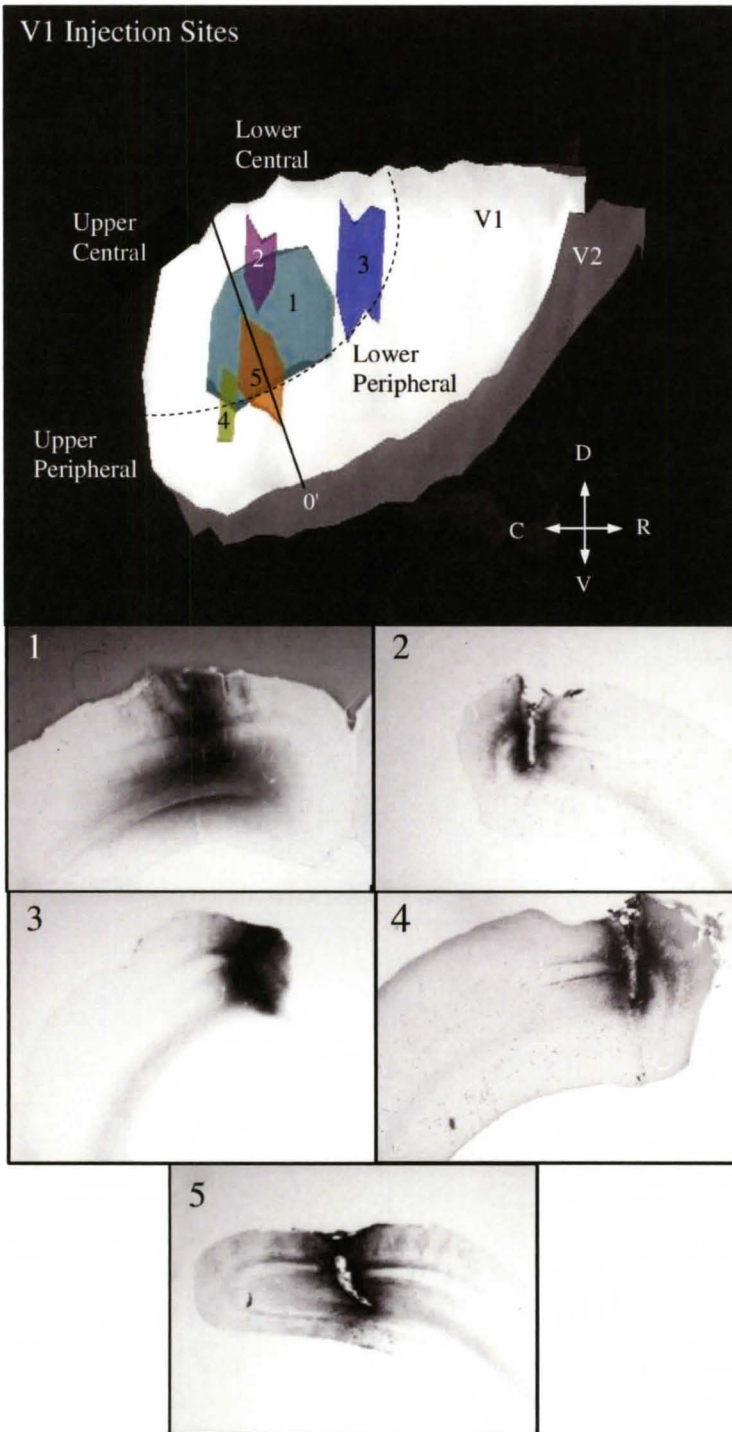


Figure 7: Projections to V2 from V1

Patches of axons (A); magnification of axon arbors in layers 3-4 (B); magnification of layer 1 showing V1 projecting to layer 1 (C); 100x magnification of axons and terminal boutons (D). Scale bars as shown.

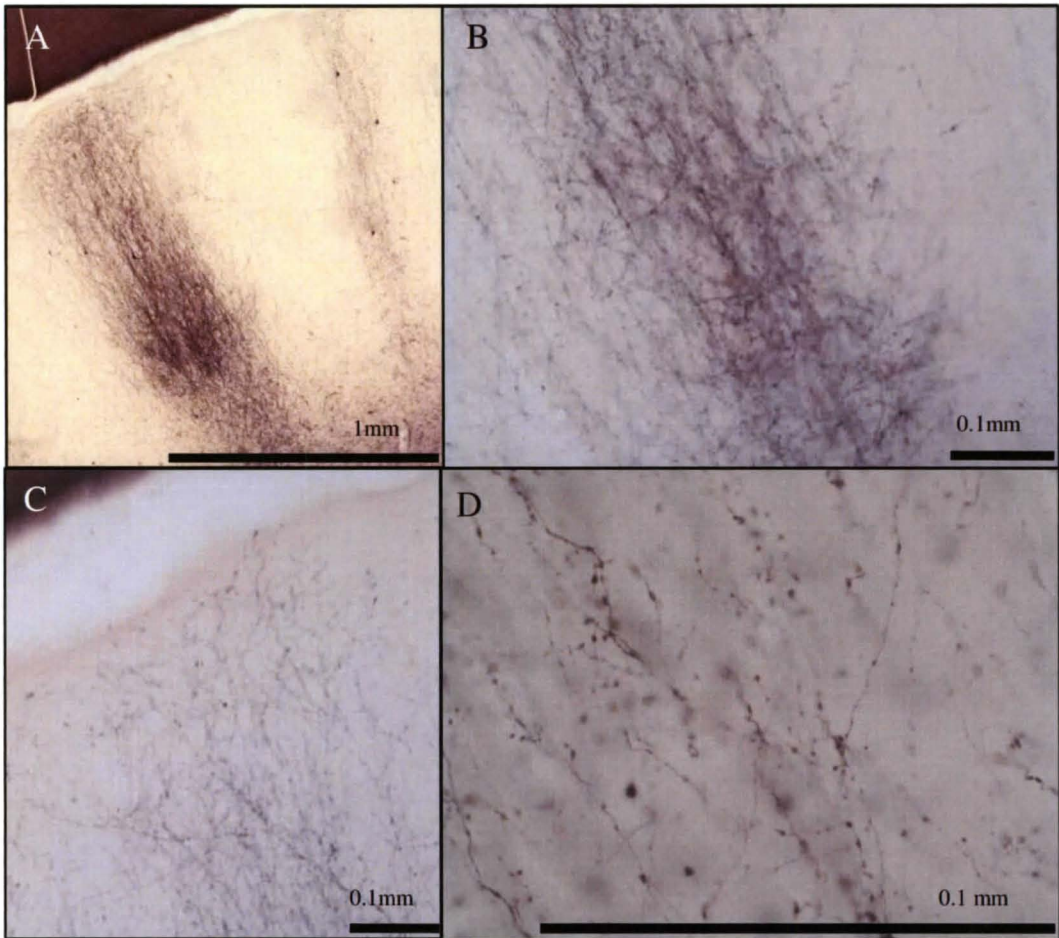


Figure 8: Projections to TP from V1 and PD

Projections to TP from V1 (A,C,E) and from PD (B,D,F). Patches of axons (A,B); CTB cell bodies in TP area (combined CTB/BDA injection) with BDA axon patch (C); magnification of layer 1 showing PD projecting to layer 1 (D); No layer 1 projections found from V1 (not shown); 100x magnification of axons and terminal boutons (E,F). Scale bars: As shown.

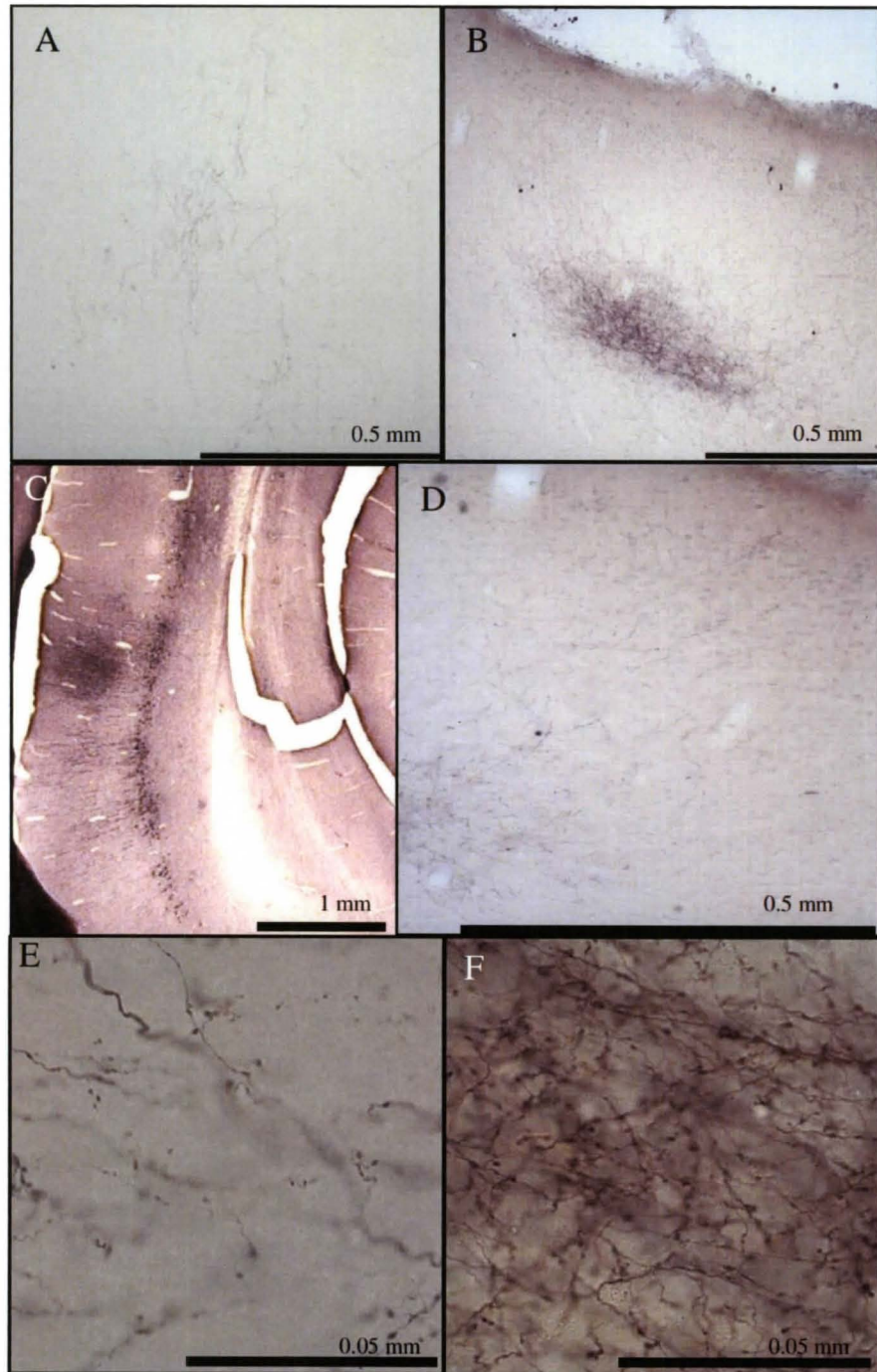
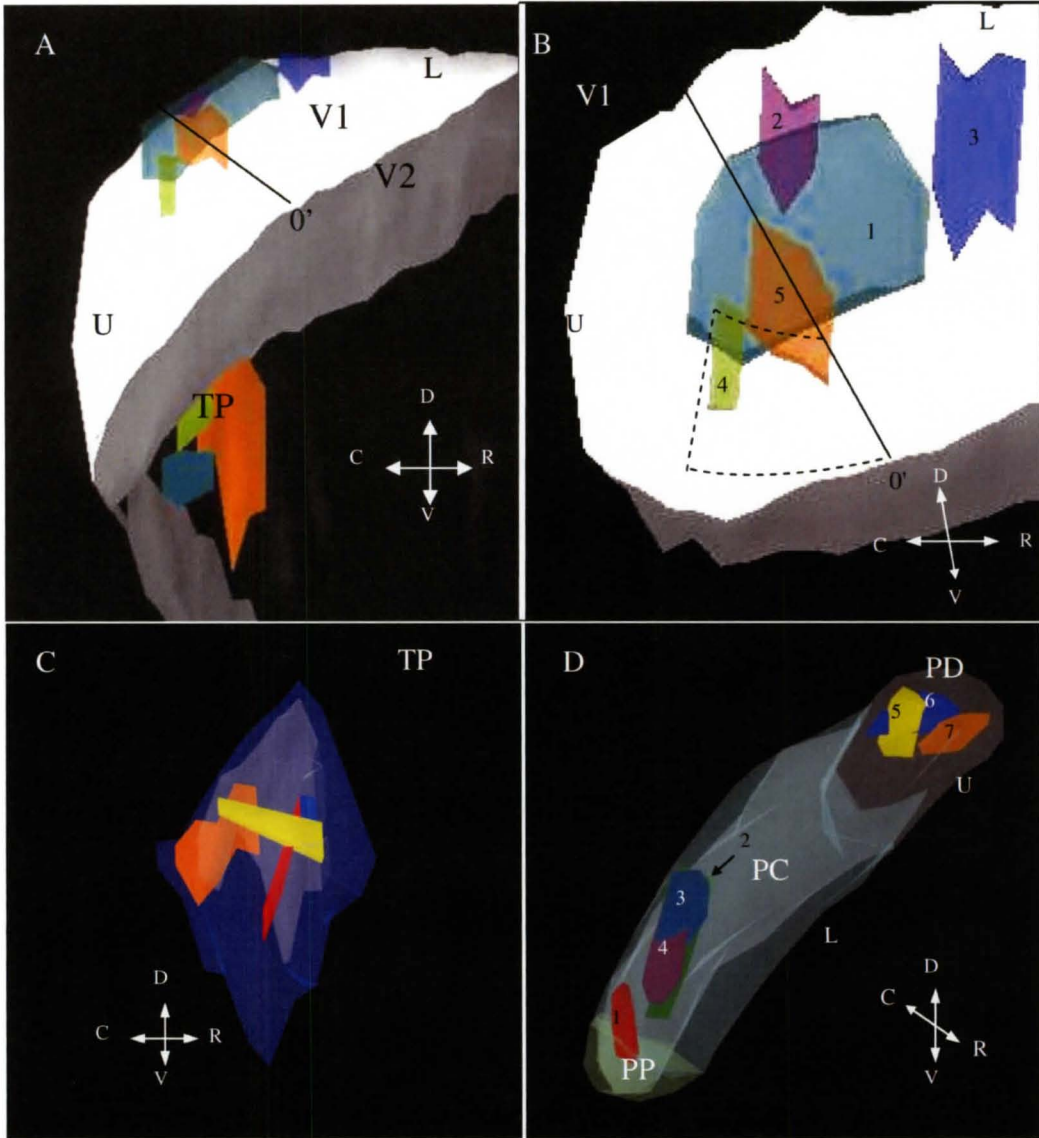


Figure 9: 3D Model of Projections to TP

A: V1 projections to TP, color coded by injection. Injection 1 – bluegreen; injection 2 - purple; injection 3 – blue; injection 4 - Injection 4 – yellowgreen; injection 5 - orange. Zero degree of the visual field shown as solid line. U = upper visual field to the left of the zero degree line. L = lower visual field to the right of the zero degree line

B: Top view of V1 injection sites. Dashed line is approximate area of V1 injections that projected to TP, based on observation of projected pattern. C: PD projections to TP, color coded by injection. Injection 1 – red; injection 5 – yellow; injection 6 – blue; injection 7 – orange. Light grey shading is the area of V1 projections to TP, showing the overlap with PD projections to TP. D: PD (5,6,7) and PC (1 – red; 2 – green; 3 – bluegreen; 4 – violet) injection sites.



Using combination CTB/BDA injections (Fig. 8F), our experiments confirm the findings of Sesma et al. (1984) that injections in discrete areas of V1 send projections to a focused area of TP, but receive projections from the whole TP area. Sesma et al. (1984) identified a small dorsal area of V1 to TP connections. We found that there are V1 to TP connections as well as TP to V1 connections throughout TP.

Temporal Dorsal Area (TD)

Projections from V1 to TD show a strong topographic pattern (Fig 11A-D) representing the lower vision field (Fig. 11E) in two separate projection patterns representing central and peripheral vision (Fig. 11F, G). There is considerable overlap of injection site projections, even from injections that did not themselves overlap.

Injection 4, in the upper visual field of V1 did not project to TD. The remaining injections, all of which are at least in part within the lower vision field did project to TD. Two regions of V1-TD axon projections can be seen clearly with the injections in figures 11B (injection 3) and 11D (injection 5). These regions appear to be representative of the lower central (area "a" in Fig. 11F) and lower peripheral (area "b" in Fig. 11F) vision fields, based on the pattern of projections. Injections 1-3 and 5 all covered part of the lower central vision field. These injections projected to area "a" of TD as seen in Figure 11F. Injections 1 and 5 also covered part of the lower peripheral vision field. These injections and only these injections projected to area "b" of TD as seen in Figure 11F. Area A of TD shows a topographic layout with the most caudal injections projecting to the most caudal parts of TD-A.

With only two injections projecting to area TD-B, topography can be seen roughly but cannot be determined. It is probable this area is also topographic, given that the projections are a topographically determined subsection of the visual field in V1.

V1-TD axon patches have a very regular, linear columnar arrangement (Fig. 12A). With injection 1 from V1, the large area of tracer allowed this pattern to be observed, as patches of tracer crossed several coronal sections at a time. The 3-D representation in figure 12 is the combined results of all 5 injections.

In area TD, corticocortical axons originating from V1 (V1-TD) display axon arborization and termination patterns similar to that observed for V1-V2 axons (Fig. 10). Both projections exhibit long straight axons distributed in tight vertical columns from layer 6 to layer 1 with collaterals spreading in a narrow horizontal area. Like V1-V2 axons, the patches of V1-TD axons are interconnected. One significant difference between the V1-V2 axon collaterals and V1-TD axon collaterals is that V1-V2 axons travel straight up from the white matter into V2, while the V1-TD axons travel diagonally from the white matter into TD. V1-V2 axons are also a mix of thick and thin axons and exhibit less branching than V1-V2 axons. Both V1-V2 and V1-TD axons give rise to large boutons, though in both areas the majority of boutons are small-to-medium sized. Like V1-V2 boutons, the V1-TD boutons are a primarily en passant with some terminal boutons. Both V1-V2 and V1-TD axons terminate in all layers (1-6) with the densest distribution found in layer 4.

Figure 10: Projections to TD from V1 and PD

Projections to TD from V1 (A,C,E,G) and from PD (B,D,F,H). Patches of axons (A,B); magnification of projections to layer 1 (C,D); magnification of layer 4 (E,F); 100x magnification of axons and terminal boutons (G,H). CTB cell bodies (CTB/BDA combined injection) in TD projecting to V1 (I). Scale bar in D is for D and G. Scale bar in E is for E and H. Scale bar in C is for C and F. All others as shown.

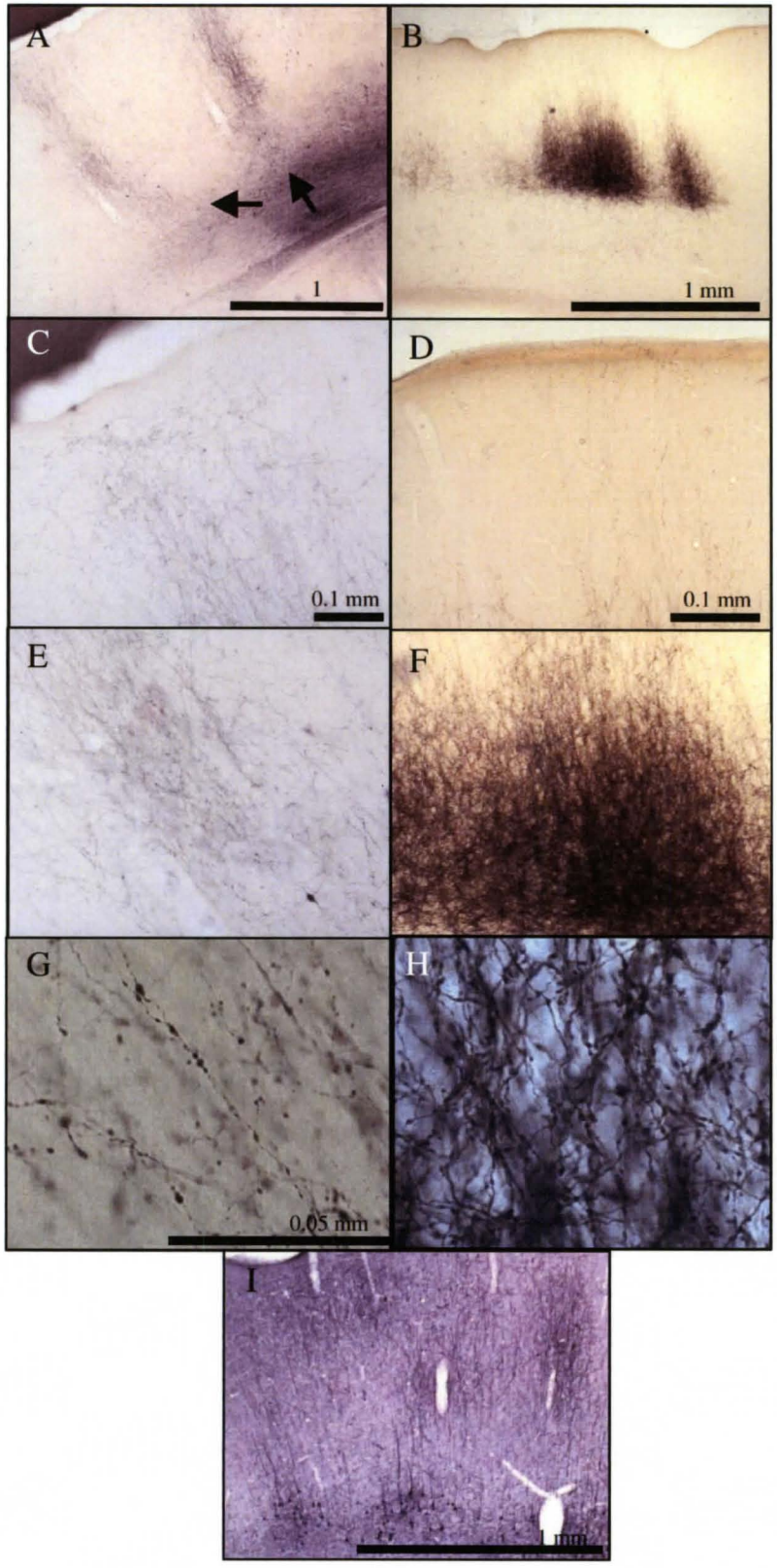


Figure 11: 3D model of projections to TD from V1

3D model of projections to TD from four V1 injections (A-D). A: Injection 2 – violet. B: Injection 5 – orange. C: Injection 1 – bluegreen. D: Injection 3 – blue. E: Combined view of the four V1 projections to TD. F: Close-up of combined view of the four V1 projections to TD showing two distinct patterns of projection in two areas: (a) from central and (b) from peripheral visual field areas of V1 (G). G: V1 injection sites. Solid line is the zero degree line of the visual field. U = upper visual field. L = lower visual field. Dotted line is between central and peripheral visual fields.

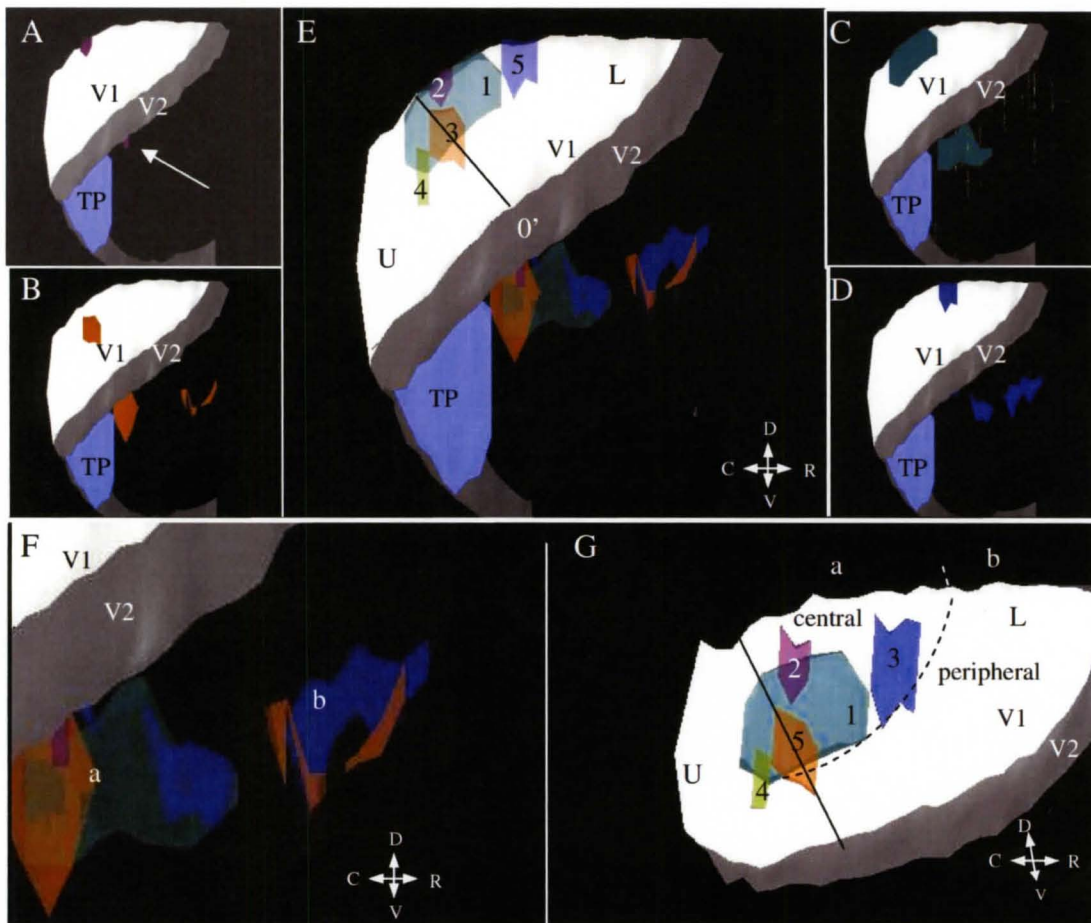
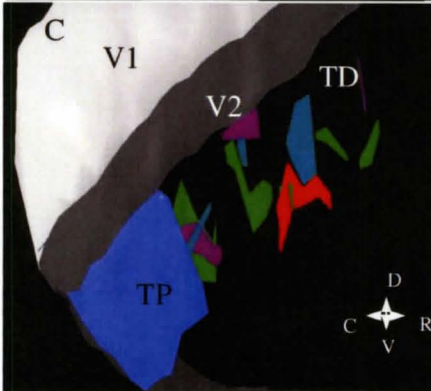
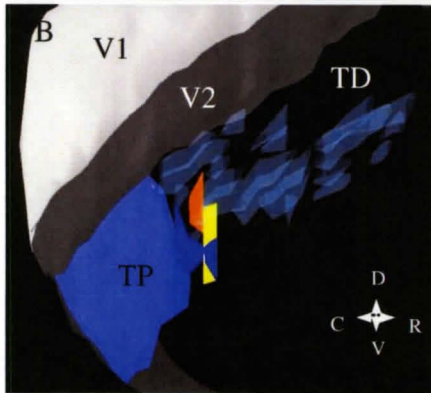
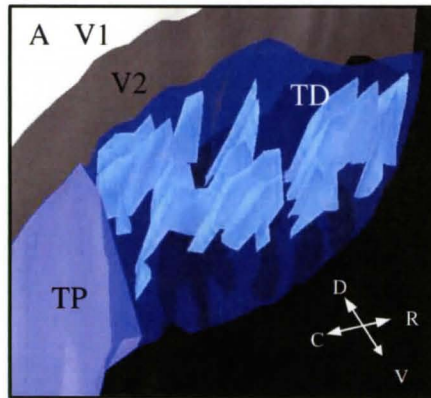


Figure 12: V1, PD and PC Projections to TD

A: Columnar pattern of axon projections from V1 to TD. B: PD projections to caudal TD mapped next to V1 projections. C. PC projections to TD.



As is seen in V2, BDA/CTB combination injections show that projections from V1 to TD are reciprocal. These injections also show that, like TP, portions of TD that do not receive projections from a specific area of V1 often still project back to that area.

Temporal Anterior Area (TA)

Projections from V1 to TA do not appear to be topographic (Fig 14). The injections which projected to TA all projected to the same broad area. Two injections did not project to TA, the furthest caudal injection and the furthest medial.

TA projections from V1 (V1-TA; Fig. 13) form interconnected patches approximately 0.5 mm wide. There are many long straight axons in a patch, but there is also extensive branching and horizontally extending axons creating a bushy appearance. The most dense axons and terminals are in layer 4. Axons do not project beyond layer 3. The axons caliber is mixed with thick and thin axons. The area is more dominated by large and medium boutons than other temporal lobe visual areas analyzed. The boutons are a mix of boutons en passant and terminal boutons, being somewhat more dominated by boutons en passant. A joint CTB/BDA injection shows that the projections from V1 to TA are reciprocal.

Temporal Inferior Area (TI)

Projections from V1 to TI do appear to be topographic (Fig 16). Two injections did not project to TI, the same two that did not project to TA: the furthest caudal injection and the furthest medial. Axons from V1 projecting to TI (V1-TI; Fig. 15) are sparse and very straight. They are thick and have few, small boutons, primarily terminal boutons. There is little to no branching of these V1-TI axons. Like V1-TD axons, V1-TI axons also come diagonally from white matter. In our experiments we did not see axons reach past

layer 3. Boutons were found in layers 3 and 4. A combined CTB/BDA injection shows that the connections are reciprocal.

Inferior Temporal Caudal Area (ITc), Inferior Temporal Intermediate Area (ITi), Inferior Temporal Rostral Area (ITr) and the Posterior Parietal Caudal area (PPC)

There are no projections from V1, PD or PC to ITc, ITi or ITr. However, there are extensive projections from these areas to V1. There are also projections to V1 from a small cortical area ventral to rostral TD and TA, rostral to TI (not shown). This area is identified as the posterior parietal caudal area (PPC) by Wong and Kaas (2009).

Figure 13: Projections to TA from V1 and PC/PP

Projections to TA from V1 (A, B, D, F,) and from PC/PP (C, E, G). Patches of axons (A, C); magnification of layer 4 (D, E) ; 100x magnification of axons and terminal boutons (F, G). CTB cell bodies (CTB/BDA combined injection) in TA projecting to V1 (B). Cell bodies in A and D are a result of tracer invading the V1 white matter. Scale bars as shown.

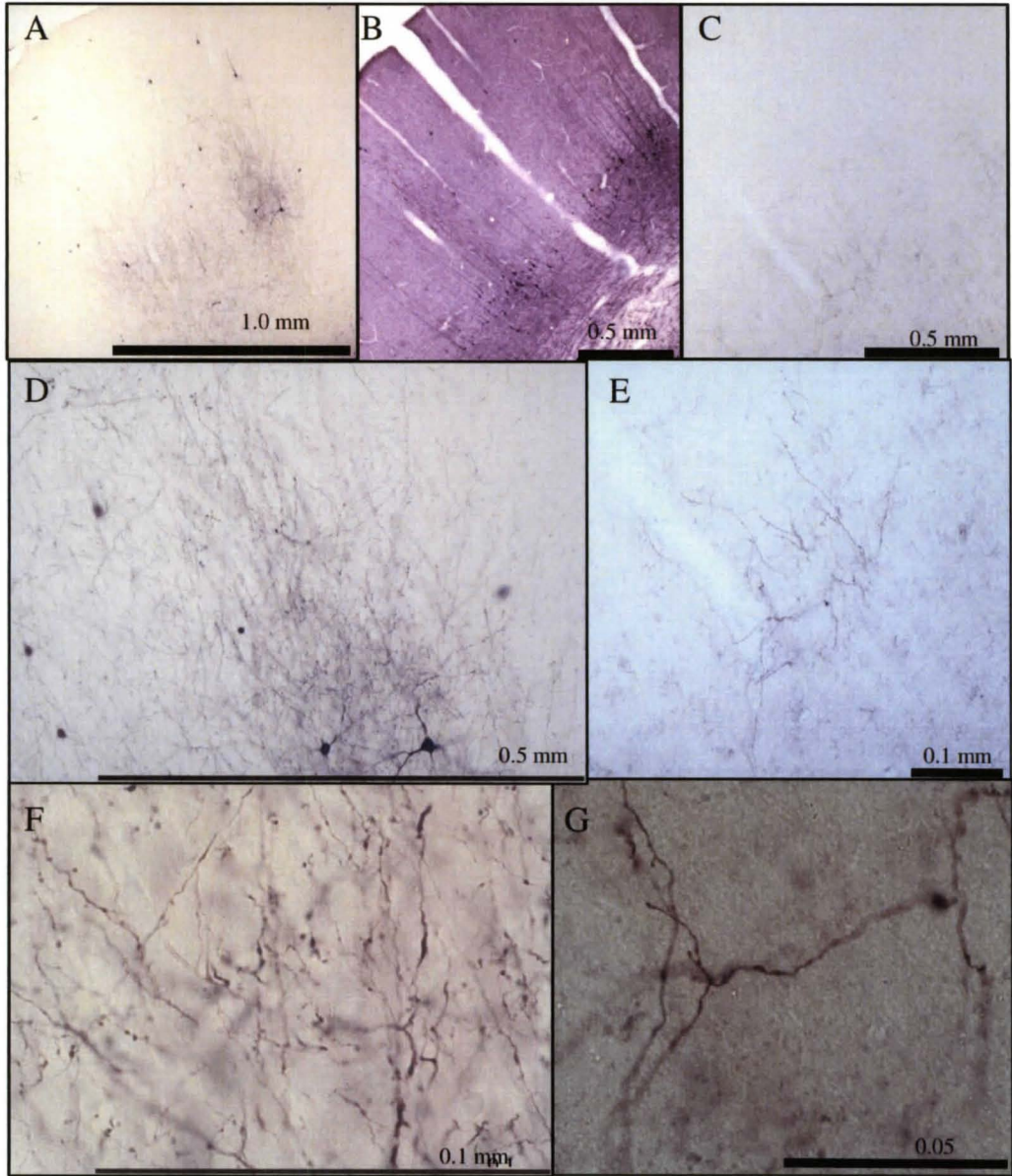


Figure 14: 3D model of projections to TA from V1 and PC/PP

3D model of projections to TA from three V1 injections (A-C). A: Injection 5 – orange. B: Injection 1 – bluegreen. C: Injection 3 – blue. D: Projections to TA from PC/PP. Injection 4 – violet; injection 1 – red. E, F: Pulvinar nucleus injection sites. Injection 1 – red; injection 2 – green; injection 3 – bluegreen; injection 4 – violet; injection 5 – yellow; injection 6 – blue; injection 7- orange.

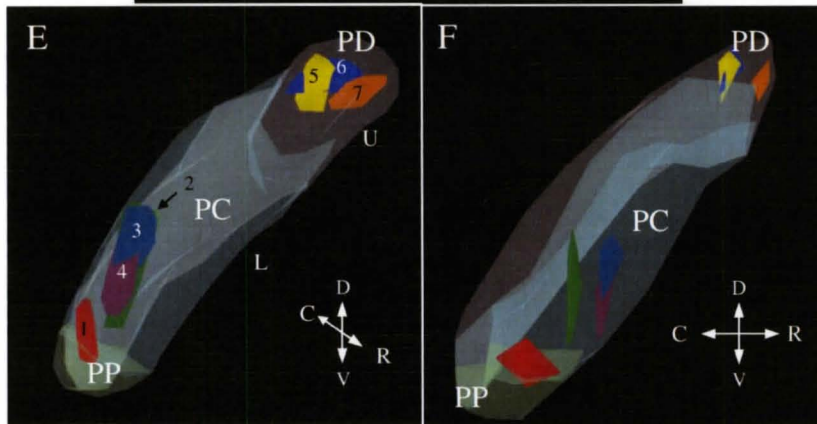
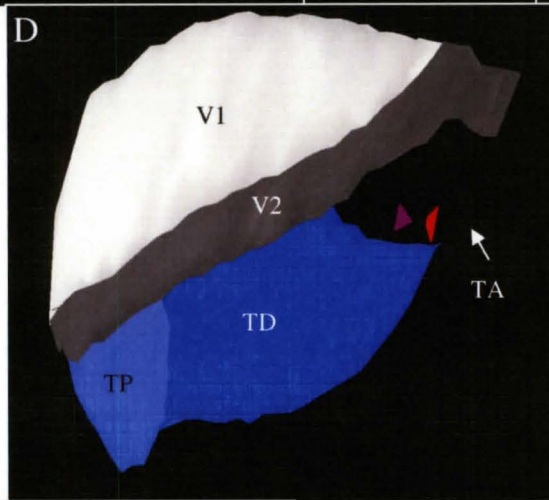
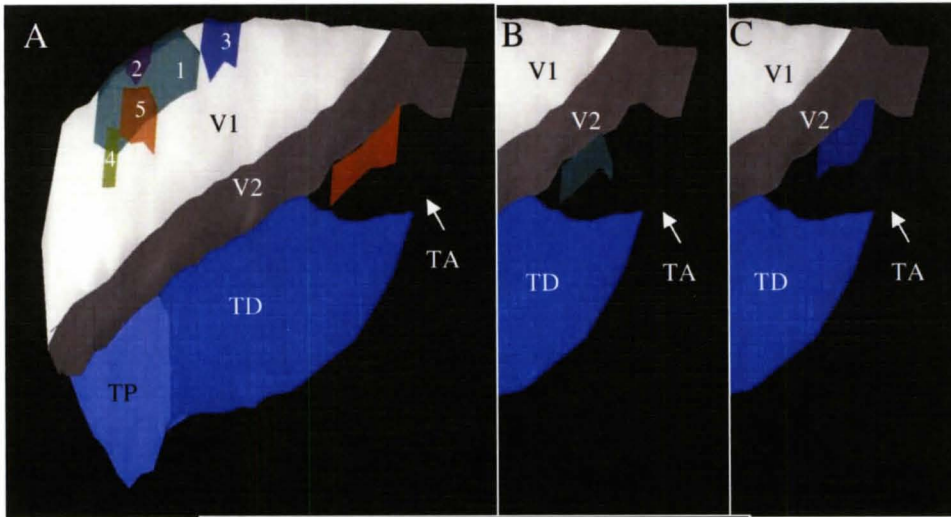


Figure 15: Projections to TI from V1 and PC/PP

Projections to TA from V1 (A, B, F,) and from PC/PP (D, E, G). Patches of axons (A, D); magnification of layer 4 (B, E) ; 100x magnification of axons and terminal boutons (F, G). CTB cell bodies (CTB/BDA combined injection) in TI projecting to V1 (C). Scale bars as shown.

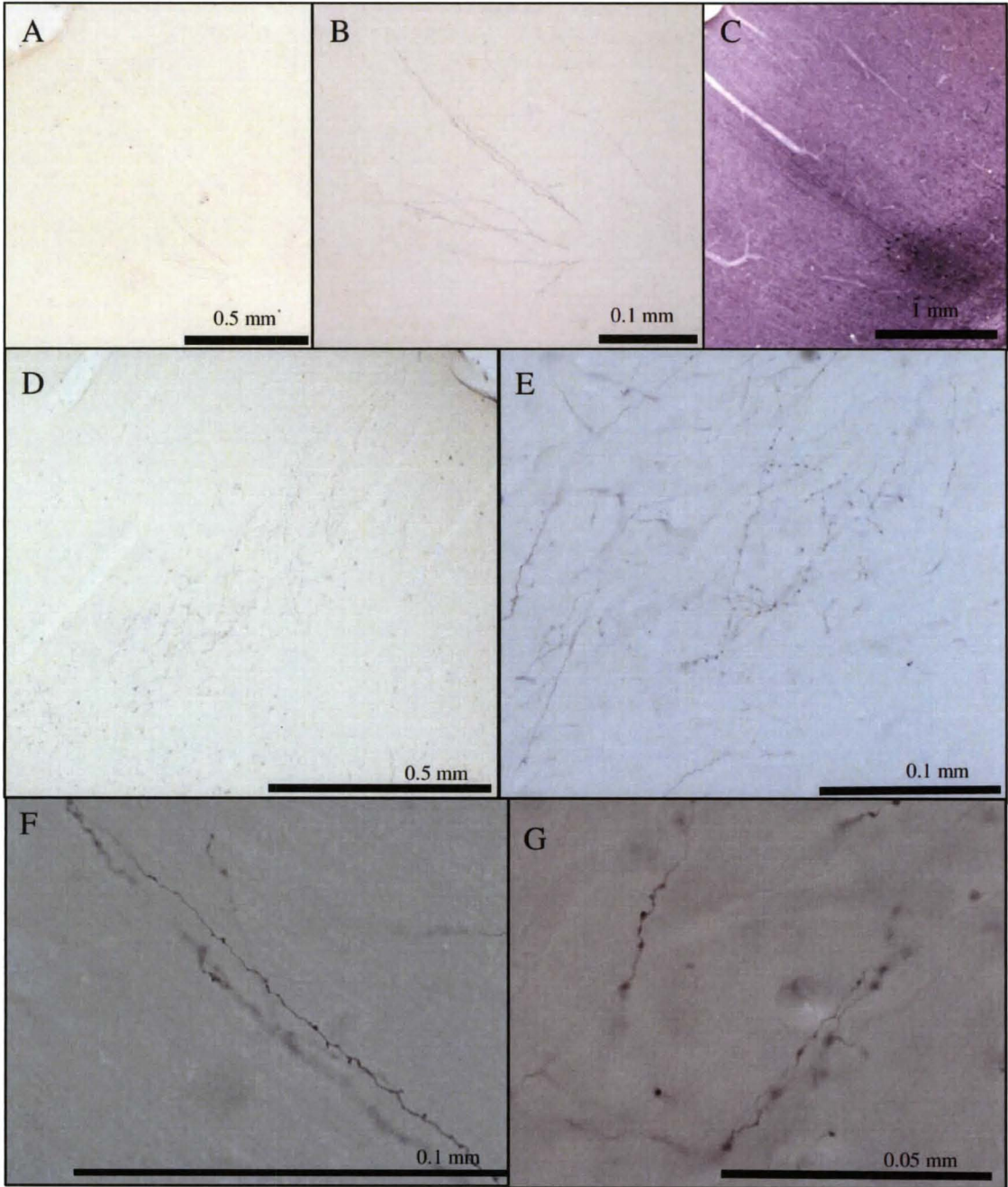
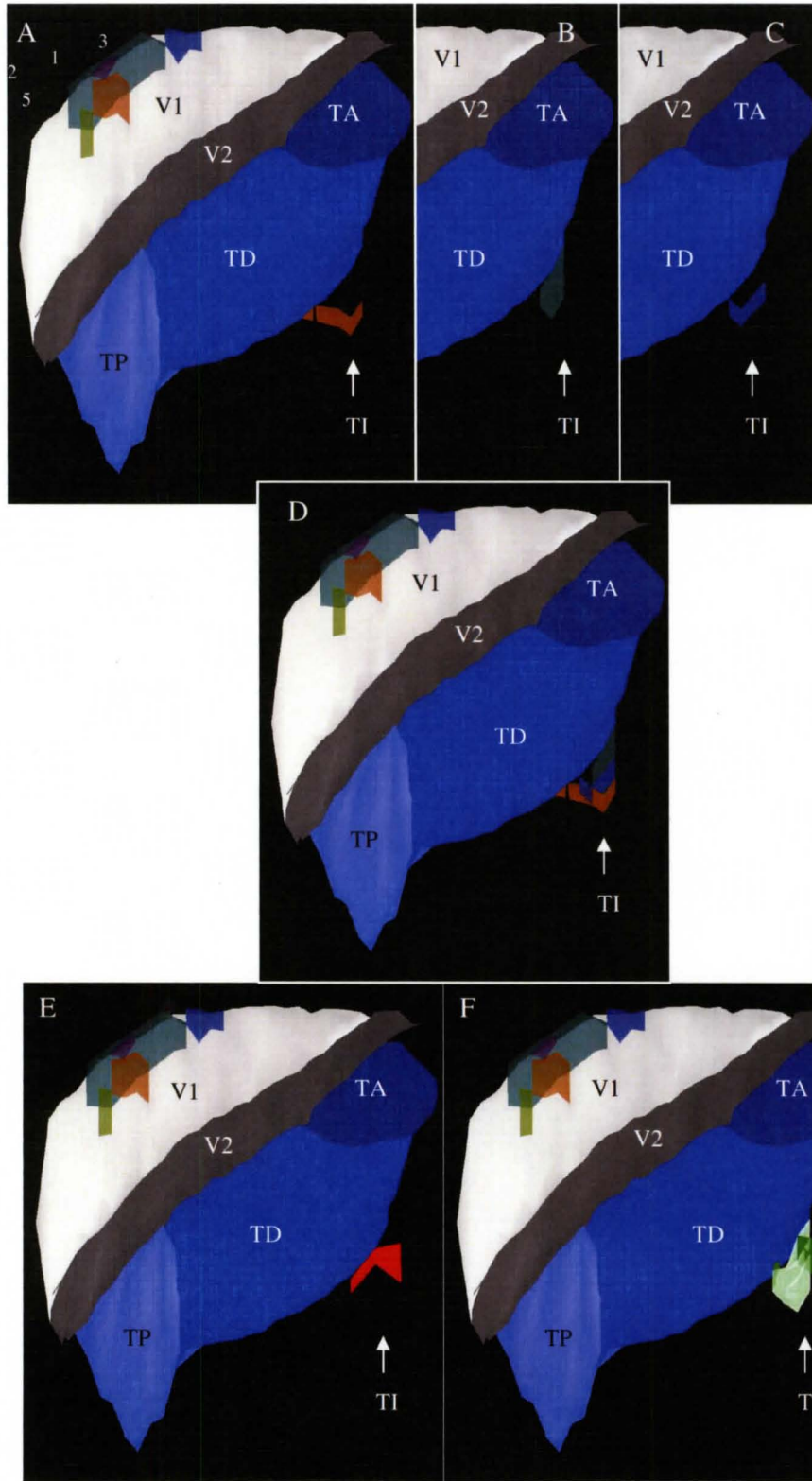


Figure 16: 3D model of projections to TI from V1 and PC/PP

3D model of projections to TI from three V1 injections (A-C) A: Injection 5 – orange. B: Injection 1 – bluegreen. C: Injection 3 – blue. D: Injections 1,3 and 5 combined. E: Projections to TI from PC/PP (Pulvinar nucleus injection 1, Fig. 13). F: Projections from V1 (light green) overlain by projections from PC/PP (dark green; F).



Pulvinar Nucleus (PD and PC) Projections

Seven injections in the pulvinar nucleus - three in the dorsal pulvinar (PD), one that hit both the central pulvinar (PC) and the posterior pulvinar (PP) and three in PC - were used to trace connections from those two pulvinar areas to locations in the tree shrew temporal lobe. These injection sites are illustrated in Figure 17.

The PP division of the tree shrew pulvinar was proposed by Lyon et al. (2003) because of distinctly different connection patterns at the posterior pole of PC than in the rest of PC. These unusual connections were rostral to the connections made by PC as a whole, such as the temporal inferior area (TI). In our research we found the same pattern. Only our extreme posterior PC injection projected to TI. Therefore, we included PP in our analysis for completeness.

Temporal Posterior Area (TP)

Axons labeled by injections in PD projected to TP, but axons labeled by injections into PC did not, with the exception of axons labeled by the PC/PP injection (Fig.9 C,D). This is consistent with our V1 injections, which showed that TP is a representation of the upper vision field. The PD projections show a generally topographical pattern. We see in figures 10C and D that the most rostral injection (7) in PD labeled axons in the most caudal regions of TP while caudal PD injections (5 and 6) labeled axons in the most rostral region of TP. Projections from PD overlap projections from V1 (light gray area in figure 9C) extensively. Additional studies could determine if they synapse on the same or different neurons in this area.

PD-TP axon projections are much denser than V1-TP axon projections (Fig 9B,C,E). The PD-TP axon patches are also much wider, and terminate almost

exclusively in layer 4. The PD-TP axons run horizontal to the white matter with an interlacing network of axon collaterals creating a net-like effect. A small number of PD-TP axons travel to layer 1 from each end of the patch. The axons are thin with mostly small-sized terminal boutons with some en passant boutons. There are a greater number of boutons, and the boutons are more clustered in the PD-TP axon projections than in the V1-TP axon projections. PD-TP axon projections have the largest concentration of boutons found in our study.

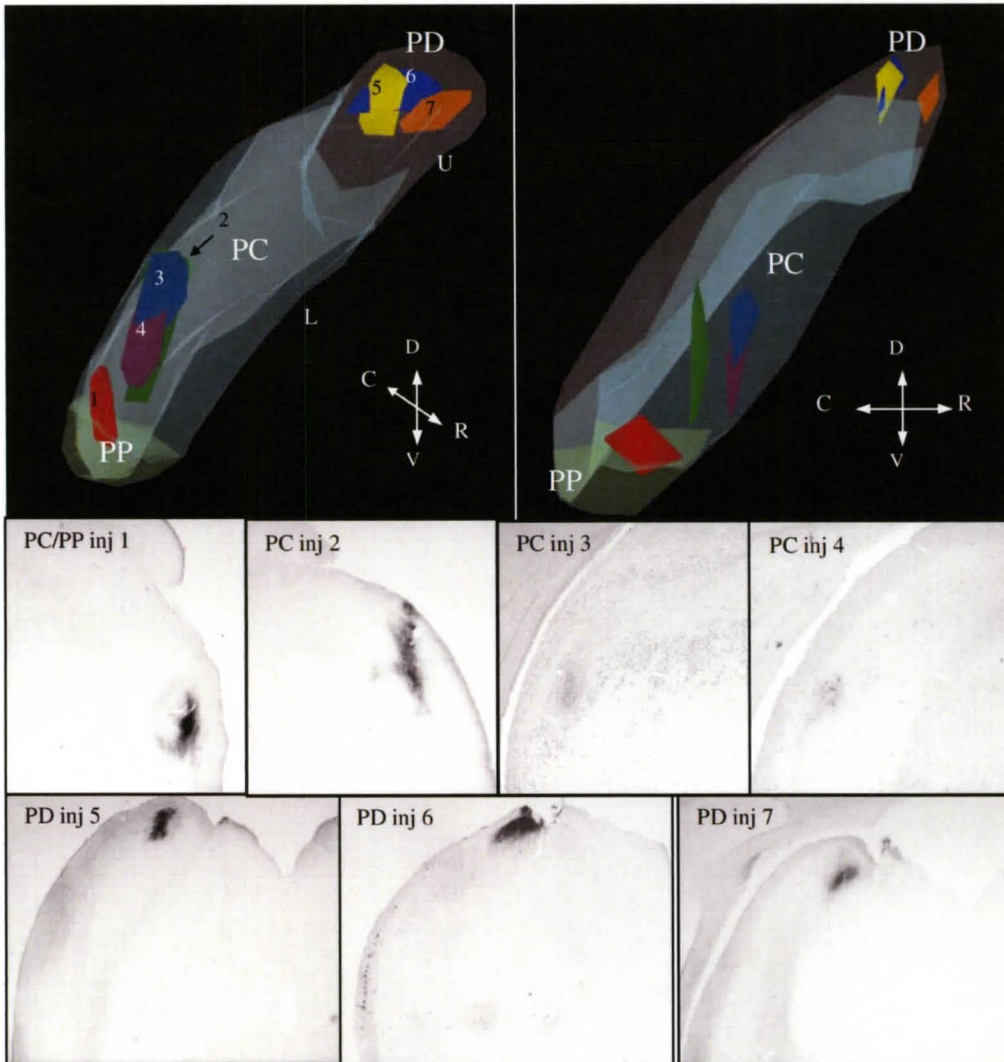
Temporal Dorsal Area (TD)

PD projects to the most caudal portion of TD (Fig 12B). There was some overlap with our V1 injections, but very little. PC projects over the whole area TD (Fig 12C). The projection pattern indicates a dual projection from PC. This dual projection can be seen as a distinct break in the projection pattern in figure 12C. This break divides TD into two regions, but the division is different than that found with V1 projections.

PD-TD axon projections produce broad patches of long straight axons heavily branched in layers 3 and 4 (Fig. 10). They appear less like columns and more like wide, interconnected patches with axons extending out of them to layer 1. The axons synapse in layers 1- 4. The axons are a mix of thin and thick axons with many medium sized as well as small boutons. The boutons are primarily boutons en passant.

Figure 17: Pulvinar Nucleus Injection Sites

PC/PP injection 1 at A2.0, DV7.0, ML-3.8 (10-04-1AB right); PC injection 2 at A2.3, DV6.5, ML -3.8 (case 8-06-1D right); PC injection 3 at A2.6, DV6.2, ML4.0 (case 4-07-11left); PC injection 4 at A2.6, DV6.0, ML3.8 (case 8-07-6 left); PD injection 5 at A2.5, DV5.0, ML2.2 (case 8-06-1D left); PD injection 6 at A2.5, DV4.8, ML2.0 (case 5-06-6 left); PD injection 7 at A3.0, DV5.1, ML1.8 (case 9-04-1AB left)



PC-TD axons projecting to the two different areas of TD are different morphologically (Fig. 18). The first type of PC-TD axon (PC-cTD) is found in the caudal area of TD where PD also projects. These axon projection patches are broad like the PD-TD axon projection patches, but are short and bushy. They branch extensively and densely in layer 4. The PC-cTD axons, unlike PD-TD axons, do not reach layer 1. The PC-cTD axons are of mixed thin and thick caliber with fewer boutons than the PD-TD axons. PC-cTD boutons are small to medium sized. The boutons are mixed boutons en passant and terminal boutons.

The second area of PC projections is the remaining more rostral area of TD (PC-rTD). In this area the projections form narrower, more isolated patches with long axons that reach layer 1. These axons primarily synapse in layers 3 and 4, but they also synapse in layers 1 and 2. PC-rTD axons are a mix of thin and thick axons with many small to medium boutons. The boutons are of mixed boutons en passant and terminal boutons. The axons have moderate branching in layers 3 and 4.

Temporal Anterior Area (TA)

PC projections to TA did not overlap VI projections (Fig 14). Only two of the 4 PC injections projected to TA. Both were at the far ventral end of PC. One of those was the injection that partially invaded PP.

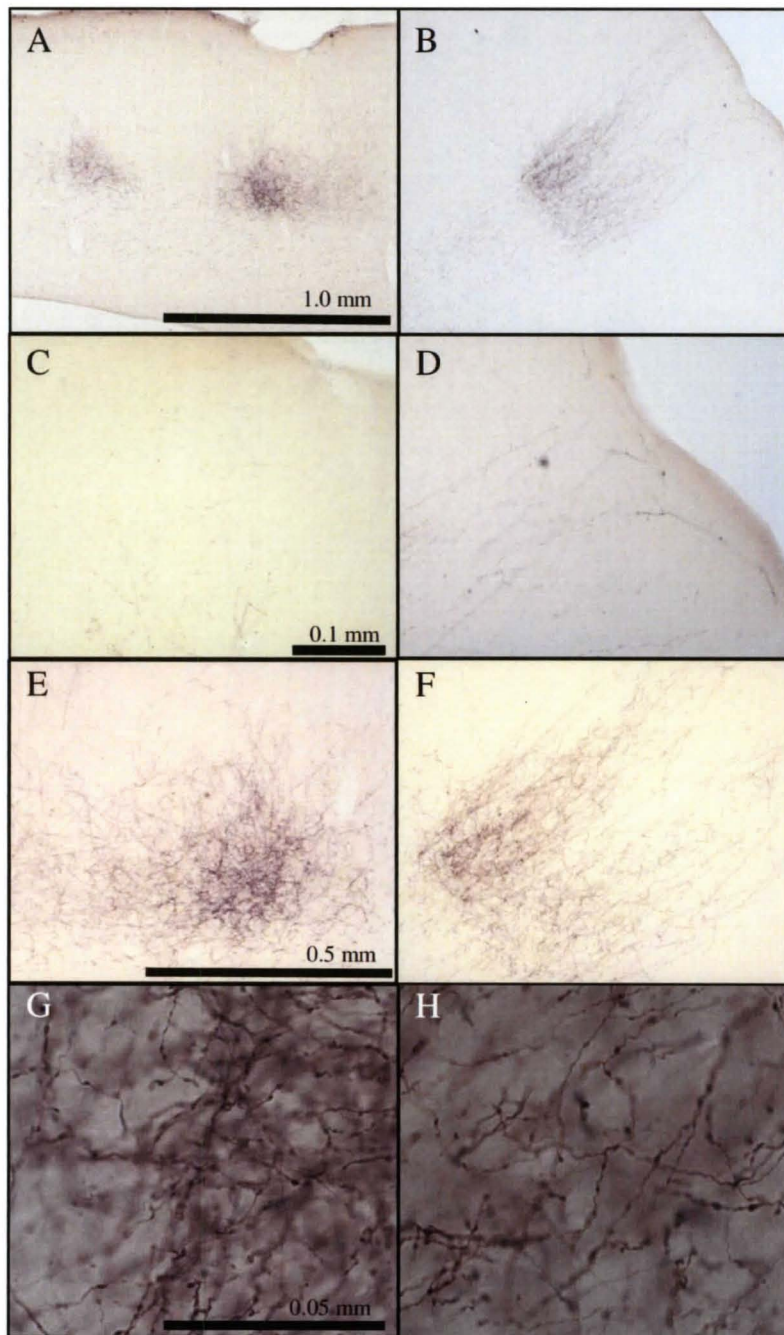
PC/PP projections to TA are sparse (Fig 13) with a moderate amount of branching covering wide patches. The axons are long and winding, predominantly remaining in layers 4-6. The tracer injections did not show axons above layer 3. The axons are thick with medium and large sized boutons, primarily in layer 5. The boutons are boutons en passant.

Temporal Inferior Area (TI)

Only the one injection that partially invaded PP projected to TI (Fig 16E). The PC/PP projection to TI overlaps V1 projections heavily. There is a general vertical direction to the pulvinar axons projecting to TI (Fig 15), which wind extensively. These axons are found in layers 1-6, and boutons are found primarily in layers 3 and 4. There is sparse branching without much spread horizontally. The pulvinocortical axons in TI are of fine caliber, sparse and have medium to large primarily en passant boutons.

Figure 18: Projections to two different areas of TD from PC

Projections to caudal TD (A,C,E,G,) and to rostral TD (B,D,F,H). Patches of axons (A,B); magnification of projections to layer 1 (C,D).; magnification of layer 4 (E,F) ; 100x magnification of axons and terminal boutons (G,H). Scale bars: A and B;C and D;E and F;G and H.



CHAPTER 4:

DISCUSSION

Defining Cortical Areas: Architectonics and Connections

Since Brodmann (1909) identified his cortical areas at the beginning of the last century using staining techniques to highlight different architectonic features of the tissue, scientists have used a variety of methods to locate distinct functional areas of the cerebral cortex. Histochemical staining not only highlights differences in the neuron size, packing and other anatomical features, it also allows scientists to investigate chemical differences. However, this method of identifying areas is not conclusive and has been subject to many disagreements and criticism (Kaas 1987; Kemper 1984).

Early fiber stains have been supplemented with a wide variety of histochemical and immunocytochemical markers that reveal more functionally relevant architectonic features. Since neurons need different chemicals to carry out different activities, architectonic immunocytochemical techniques can elucidate real functional variations (Diamond, Fitzpatrick et al. 1993). Still it is not conclusive to identify an area architectonically. Connectivity patterns are important characteristics of a functional cortical area (Lyon and Kaas 2001). An individual area should have a systemic pattern of connection, such as a sensory map that is different in adjoining cortical areas (Kaas 1987). There is a wide range of techniques for identifying connections between areas. In

this study we have used the pattern of visualized axons resulting from the anterograde transport of injected tracers.

Cortical areas are often not separated by hard lines, rather they may transition slowly or exhibit cells that stray outside the defined area. The model brain used in this study is our best fit model using both architectonics and connectivity patterns from this and past studies (Chomsung et al. 2010; Wong and Kaas 2009; Lyon et al. 1998,2003a,2003b; Jain et al. 1994; Diamond, Fitzpatrick et al. 1993; Sesma et al. 1984) to delineate the temporal lobe areas of the tree shrew involved in vision.

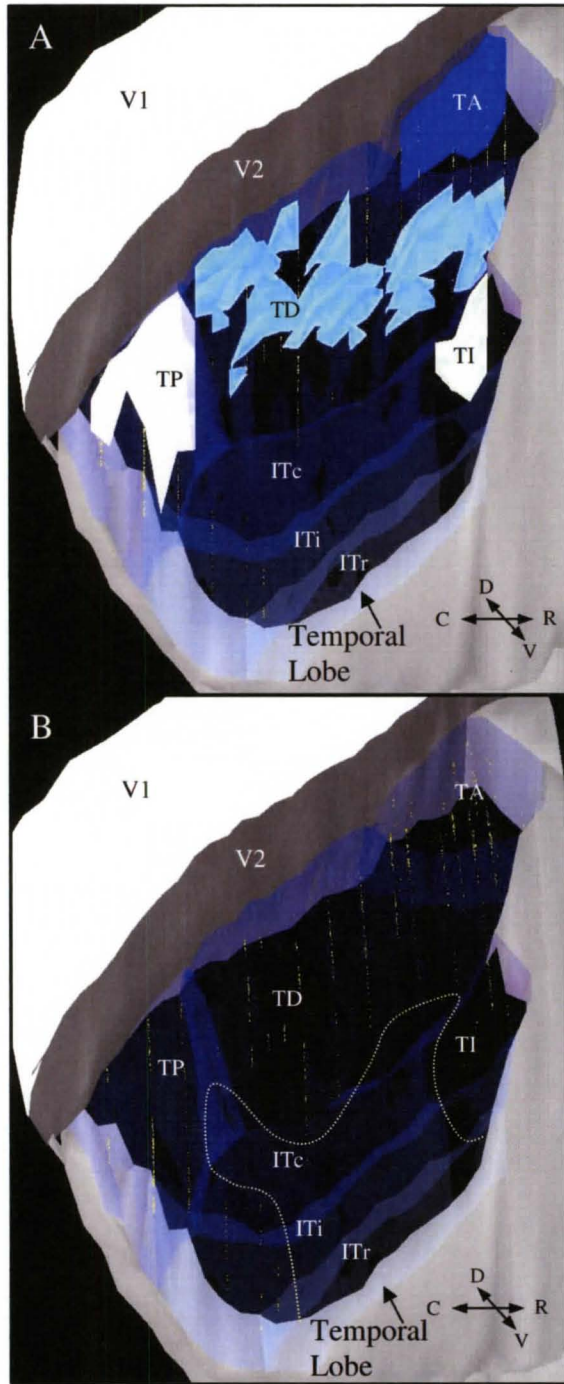
We found some variations between architectonics and connectivity patterns. In both our experiments and those of Sesma et al. (1984) the projections from V1 to TD did not reach as far as the architectonic ventrorostral border. However, projections from PD and PC did cover the far ventrorostral region of TD. A more extensive map of V1 projections could uncover whether there are V1-TD connections in the TD ventrorostral region, or if this is an area that only receives secondary pathway projections.

Using WFA and Parv staining, the location of TI was unclear. But in combining myelin staining and V1-TD, PC-TD, V1-TI and PC/PP-TI projection patterns, our experiments showed that area TD borders area TI (Fig. 12, Fig. 16 and Fig.19A). This was not the finding in Wong and Kaas 2009 using architectonic techniques.

Axon projections from the temporal lobe back to V1 from our one large combined CTB/BDA V1 injections cover a large portion of the temporal lobe and provided additional data in defining the area boundaries (Fig. 19B).

Figure 19: 3D model of projections between V1 and the Temporal Lobe

3D model of projections between V1 and the Temporal Lobe. A: Projections from V1 to TP (white on left), TD (light blue), TA (blue), TI (white on right) with translucent overlay of area map (translucent lines), and an underlay of the locations of cell bodies that project axons to V1 (yellow dots). B: Translucent overlay of area map (translucent lines) with the locations of cell bodies that project axons to V1 (yellow dots). Cell body area is outlined in a yellow dotted line.



Secondary Visual Pathway Temporal Cortical Areas

As we have seen from the projection data above, TP and TD are important cortical areas in the study of the secondary visual pathway. We found, as did Chomsung et al. (2010) that both PC and PD project to two focal areas in the temporal lobe. In that study, the projections were simply referred to as temporal projection 1 (T1) and temporal projection 2 (T2) because architectonics were not additionally used to define cortical areas.

The projection pattern we saw fits well with the T1, T2 projection pattern found in that study. PD projects to TP (Fig. 9C) and to caudal TD (Fig. 12B). PC projects to caudal TD and a second time in rostral TD (Fig. 11C). T1 corresponds roughly to our TP and part of caudal TD. T2 corresponds to the remaining portion of TD.

Our V1 projections to TP and TD confirmed the findings of Lyon et al. 1998 that V1-TP projections are not as dense as V1-TD projections, and V1-TA projections are sparse. Our TP projection pattern correlates to findings by Lyon et al. 2003. That research found that PD projects to TP, but PC does not. They also found that PP projected to a temporal posterior inferior area (TPI) that we found from our mapping probably included ventral TP where we see the PP projections.

In their research, Lyon et al. (2003) did not find any projections from PC to TA. Their findings showed that PP and PV projected to TA. From those findings and ours, further research would need to be done to determine if the far ventral portion of PC does project to TA, or if the projections we are seeing are from PP, even though our

micrographs of the injection site do not uncover tracer from that injection as far ventral as PP. Also in that research by Lyon et al. (2003), they found that PP and not PC projected to TI. This projection in our study is most likely a PP to TI projection.

Projections from PC (Lyon et al. 2003; Luppino et al. 1988) and PD (Luppino et al. 1988) to V2 have been previously reported. These two studies used retrograde tracer injections in V2 to identify cell bodies in the thalamus that project to V2. None of our anterograde injections in the pulvinar nucleus regions PC and PD projected to V2. In reviewing those studies, the lack of PD to V2 projections in the more recent study by Lyon et al. (2003) may indicate that the injections by Luppino et al. (1988) overran the boundary of V2 to TP (Luppino et al. 1998 figure 8) and V2 to TD (Luppino et al. 1998 figure 9), so that the cell bodies found in PD came from TP and / or caudal TD.

The cell bodies found in PC from V2 retrograde injections in Lyon et al. (2003) may likewise be an invasion of the temporal lobe areas from the V2 injections, or they could represent a group of cells that our PC injections did not contact. Further, more focused, perhaps optogenetic studies of the PC nucleus projections will be needed to resolve this issue.

During our research we found that the area TP, based on V1 projections, PD projections and the lack of PC projections, is a representation of upper vision field information. PD carries a representation of the upper half of the field of vision from the superior colliculus while PC carries a representation of the lower vision field (Chomsung et al. 2008; Dang et al. 2012). This matches data from Lyon et al. (2003), where they also found that only PD in the pulvinar nucleus projected to TP. In addition, it appears

from V1 projections to be entirely or at least primarily representative of peripheral vision (Fig. 20).

In our experiments, TD was found to have three "zones" of projection. The caudal portion of TD contains V1, PD and PC projections which have broad networks of axons and collaterals synapsing primarily in layer 4. Based on retinotopic maps of the V1, PD and PC projections, this caudal area has representations of the central upper and lower vision field. The rest of TD has projections only from V1 and PC which exhibit long straight axons in layers 1-4 synapsing in all these layers. From the V1 and PC retinotopic maps, this area appears to represent an area of central lower vision and an area of peripheral lower vision. (Fig 20).

In previous research it has been suggested that TD may be homologous to area MT, a visual motion area, in the primate (Sesma et al. 1984; Lyon et al. 1998). The tecto-recipient zones of the tree shrew pulvinar project to TD and a second area, TP, just as the tecto-recipient zones of several studied primates project to area MT and a second area that varies in location (Baldwin et al. 2011; Lyon et al. 2010; Kamishina et al. 2009; Wong et al. 2008; Paxinos 2004; Coogan and Burkhalter 1993; Glendenning et al. 1975). The evolutionary history of tecto-recipient pulvinar nucleus projections to temporal cortex is slowly being uncovered. This secondary pathway, possibly corresponding to attentional coding of visual movement may have developed different strategies in different animals, given different evolutionary needs.

We know that even rodents such as rats have a tecto-recipient area (lateral posterior thalamic nucleus caudal and lateral areas) that projects to the occipital/temporal area (Oc1, Oc2L, Oc2M; Kamishina et al. 2009; Masterson et al. 2009); however, it has

not yet been described in specifics using anterograde transport techniques (Fig. 21A). Gray squirrels have a secondary vision pathway that projects to the temporal posterior area (TP), temporal medial area (Tm) and area 18 (V2) of the gray squirrel cortex (Abplanalp, 1970; Kaas et al. 1972; Baldwin et al. 2011; Fig. 21B). This pattern of connection is very similar to what we found in tree shrews. It is likely that tree shrews and gray squirrels have similar vision needs. Both are ground foragers that are prey animals for flying predators such as hawks.

The galago secondary pathway projects to the temporal lobe in two areas: area MT and an area ventral to area MT (Glendenning et al. 1975; Fig. 21D). The macaque pulvinar tecto-recipient zone projects to MT and V3 (Lyon et al. 2010; Fig. 21E). In humans, tractography imaging studies have shown that the pulvinar projects to V1, V2, V3, V4 and V5 (Leh et al. 2008; Fig. 21F); however, specific locations within those areas of the pulvinar tecto-recipient zone projections have not yet been described.

Differences in the size and retinotopic mapping of these temporal lobe targets of the secondary pathway are likely due to evolutionary forces related to the environment and capabilities of the animal. Tree shrews are fast-moving ground foraging animals with flying predators. TP representing just the peripheral upper visual field receives input from the tecto-recipient pulvinar pathway, which contains motion sensitive cells as part of attention related coding of visual movement (Dang et al. 2012). This peripheral upper visual field could be related to flying predator awareness.

Figure 20: Projections to TP and TD from V1, PC and PD

Projections to TP (upper peripheral vision field): from PD – orange; from V1- gray. Projections to caudal TD (upper and lower central vision field): from PD – yellow; from PC – green; from V1 – blue. Projections to rostral TD (lower central and peripheral vision field): PC – green; V1 – blue.

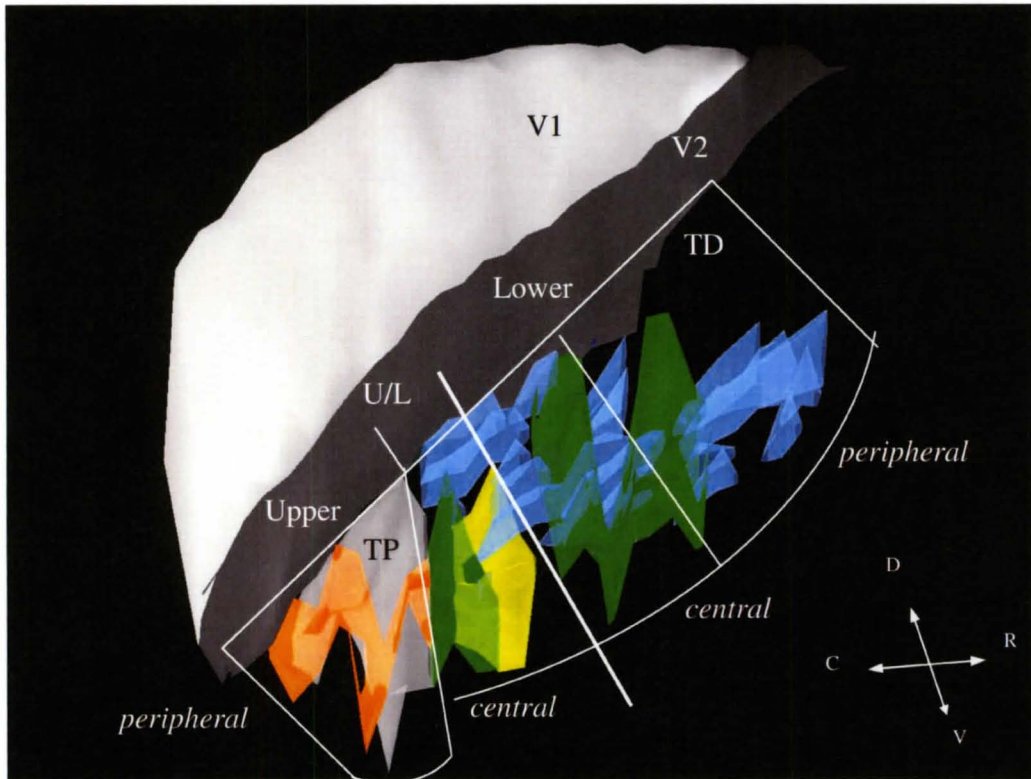
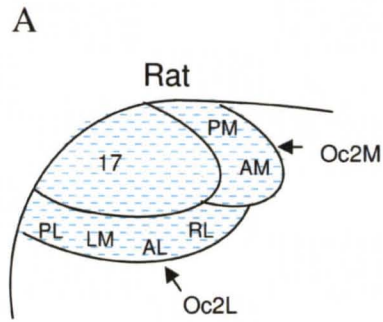
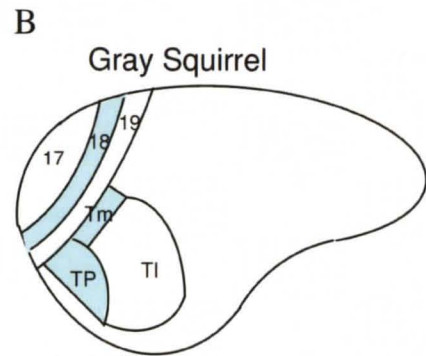


Figure 21: Secondary Pathway Projections to the Temporal Lobe

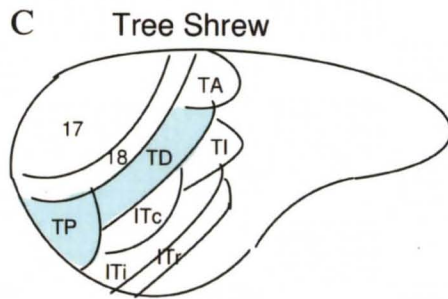
Secondary Pathway Projections to the Temporal Lobe. A: In rats, projections are to areas 17 (Oc1), Oc2L and Oc2M, but are not specifically defined within those areas. B: In gray squirrels, projections are to area 18, TP and Tm. C: In tree shrews, projections are to TP and TD. D: In galagos, projections are to MT and an area below MT not labeled in Glendenning et al. (1995). E: In macaques, projections are to V3 and MT. F. The pulvinar nucleus projects to V1, V2, V3, hV4, TO1 and TO2. In humans, tectorecipient zone projections were not distinguished from other pulvinar nucleus projections.



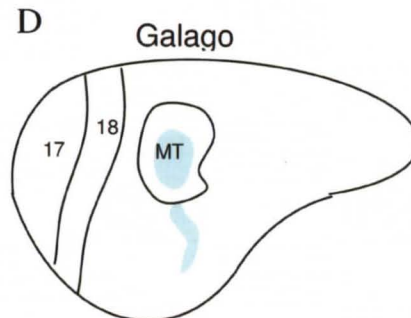
Secondary Pathway Projections (ns)
 (Coogan and Burkhalter 1993; Paxinos 2004; Kamishina et al. 2009)



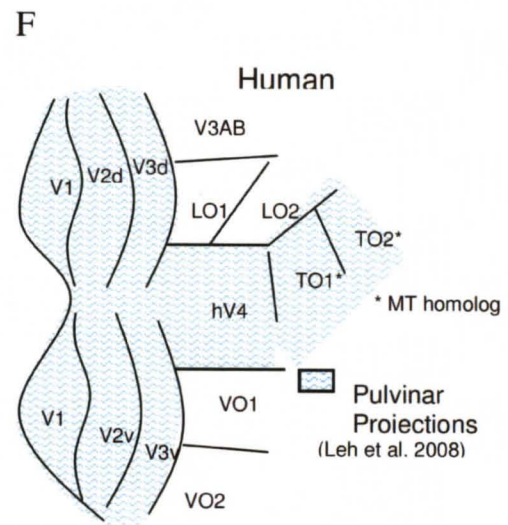
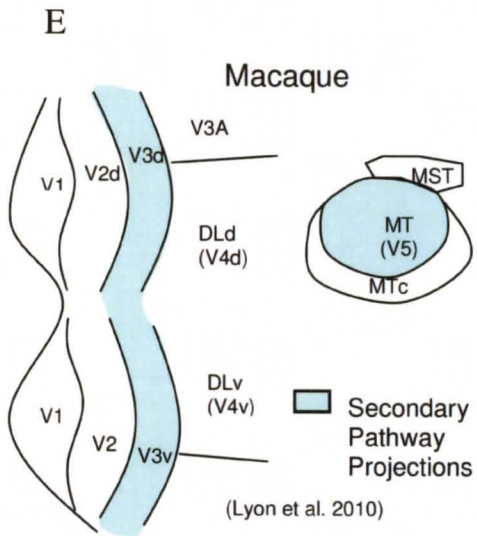
Secondary pathway
 (Wong et al. 2008; Baldwin et al. 2011)



Secondary pathway projections



Secondary pathway projections
 (Glendenning et al. 1975)



The large size of the lower vision field map in rostral TD could enable the speed of movement the tree shrew exhibits by allowing the animal to retain a large navigational map. The combination of upper and lower central vision fields in caudal TD could enable better attention to foraging in the immediate area. Ongoing behavioral studies will tell us more about the functions of these areas. Mapping the function of temporal lobe visual areas within the secondary pathway of many different animals will give us insight into evolutionary changes.

Comparing Primary and Secondary Visual Pathway Projections to Temporal Lobe Areas

The tecto-recipient zone of the pulvinar nucleus receives inputs from widefield vertical cells in the superficial layers of the SC. Widefield vertical cells respond to moving visual stimuli and are direction selective (Mooney et al., 1988). In previous studies, it has been shown that one of the output targets of the tecto-recipient pulvinar nucleus in primates (area MT) and tree shrews (dorsal temporal cortex, TD) responds to moving visual displays (Kaufmann et al., 1979; Maunsell and VanEssen, 1983; Felleman and Kaas, 1984; Allman et al., 1985; Born and Bradley, 2005).

Several studies have demonstrated that lesions or inactivation of V1 dramatically reduce activity in primate MT and tree shrew TD, suggesting that the response properties of area MT/TD are dependent on (driven by) inputs from area V1 (Kaufmann et al., 1979; Kaas and Krubitzer, 1992; Collins et al., 2003, 2005). Movement-sensitive visual signals in area MT are not completely abolished following V1 lesions (Girard et al., 1992; Rodman et al., 1989) and, because V1 densely innervates the superficial layers of the SC (where the dendrites of widefield vertical neurons are located; Boka et al., 2006;

Chomsung et al., 2008), it is still unclear whether the large reduction in MT activity following V1 lesions is primarily due to inactivation of direct (V1-MT) or indirect (V1-SC-pulvinar-MT) projections. Furthermore, neurons in the tecto-recipient pulvinar of the primate (Berman and Wurtz, 2011) and tree shrew (Dang et al. 2012) are responsive to the movement of visual displays, and therefore could relay movement selectivity to temporal cortex neurons.

In describing the function of the dorsal thalamus in the visual system, the concept of drivers and modulators was developed. “Drivers” define the response properties of the postsynaptic neuron and “modulators” can alter the strength or effectiveness of the drive without substantially affecting receptive field properties (Sherman and Guillery, 1998). For example, the receptive fields of dorsal lateral geniculate nucleus (dLGN) neurons are very similar to those of the retinal ganglion cells that innervate them while inputs from V1 alter the strength or effectiveness of their responsiveness to retinal input without substantially affecting the characteristics of their responses to visual stimuli. Likewise, geniculocortical afferents that terminate in layer 4 of area V1 are considered “driver” inputs because the receptive field properties of V1 neurons are dependent on input from the dLGN.

In the cortex, visual connections referred to as ‘feedforward’ projections from the primary visual cortex (V1) to other visual cortical areas, have been shown to terminate in layers 3 and 4 as “driver” inputs do (Lowenstein and Somogyi, 1991). ‘Feedback’ projections from those other visual areas to V1, primarily target layer 1, with layers 3 and 6 also receiving input (Rockland and Drash, 1996). Previously, it was thought that there was always one driver (feedforward) and many modulators (feedback). Recently,

theories of multiple driver types, and connection types beyond drivers and modulators have begun to be defined (Sherman and Guillery, 2011; Viaene et al., 2011; Wei et al., 2011).

In our study, all of the projections to the visual temporal cortex terminated primarily in layer 4. Often, these projections also terminated substantially in layer 3. V1 and PC/PP projections to areas TA and TI included majority terminations in layer 5 (PC/PP to TA) and layer 3 (V1 and PP to TI). However, due to the involvement of PP, these pulvinar nucleus projections do not appear to be from the tecto-recipient zone.

Our findings that both primary visual pathway (from V1) and secondary visual pathway (from the pulvinar nucleus tecto-recipient zone, PD and PC) axon projections terminate primarily in layer 4 would indicate that the connections from both pathways are "driver" or "feedforward" type. Alternatively, these results could indicate that the functional model of driver/modulator does not apply to higher level visual cortex areas.

In the thalamus, drivers tend to have large caliber, highly branching axons, fewer but larger axon terminals than modulators and synapse on proximal dendrites. Drivers also have denser, more tightly localized arbors. Modulators have thin, minimally branching axons, many small terminals and can synapse proximally or distally. These also tend to have less en passant ("bead-on-a-string") boutons and more short-stalked terminal boutons. (Sherman and Guillery, 1998,2002,2011; Guillery 1966; Roan et al., 2012).

The V1 and pulvinar nucleus tecto-recipient zone projections to the temporal cortex could not be categorized by these criteria as either drivers or modulators. The projections were for the most part thin, dense and moderately to heavily branched. The boutons were mostly small and numerous.

Similarities and differences in axon and bouton characteristics appear to group the projections more tightly by target cortical area than by axon source (Fig. 22). V1 and PD axons terminating in TP tend to create wide fields of thin axons with extensive branching and many small boutons of mixed boutons en passant and terminal bouton type. V1-TP axons are sparser than PD-TP axons and are more likely to give rise to boutons en passant.

Pulvinar nucleus projections to caudal TD (PD-TD, PC-cTD) also result in wide fields. These are dense and extensively branched with mixed thin and thick axons and many small to medium sized boutons. PD-TD axons were more likely to give rise to boutons en passant than PC-cTD axons.

V1 projections to TD (V1-TD) and pulvinar nucleus projections to rostral TD (PC-rTD) are organized in narrow local arbors of mixed thin and thick caliber axons with sparse to moderate density and branching. Boutons are small to medium sized. V1-TD axon branching is more sparse than PC-rTD axon branching. V1-TD axons have slightly more boutons than PC-rTD axons.

CHAPTER 5:

CONCLUSION

Ongoing studies of the pulvinar nucleus' role in attention (Kastner et al. 2004; Saalman et al. 2012) are investigating the functional role of cortical-pulvinar-cortical loops as well as tecto-pulvino-cortical pathways (Wilke et al. 2009,2010;Chomsung 2009,2010). This paper focused on regions of the pulvinar nucleus that receive input from the superior colliculus (tecto-pulvino-cortical pathways).

We found that by combining architectonics and morphological connection pattern analysis we could more clearly describe the key areas where the primary pathway from the retina, through the dorsal lateral geniculate nucleus (dLGN), to the striate cortex (V1) meets the secondary pathway from the retina through the superior colliculus and pulvinar nucleus. The two areas of the cortex in which these two pathways intersect are the temporal posterior area (TP) and the temporal dorsal area (TD; Fig.22). Our research indicates that it is likely that the scheme of identifying drivers and modulators as it is defined for the primary pathway from the retina to the LGN to V1 does not apply to this area of convergence. Rather, the target area defines the morphological characteristics of projections to that target area from both pathways.

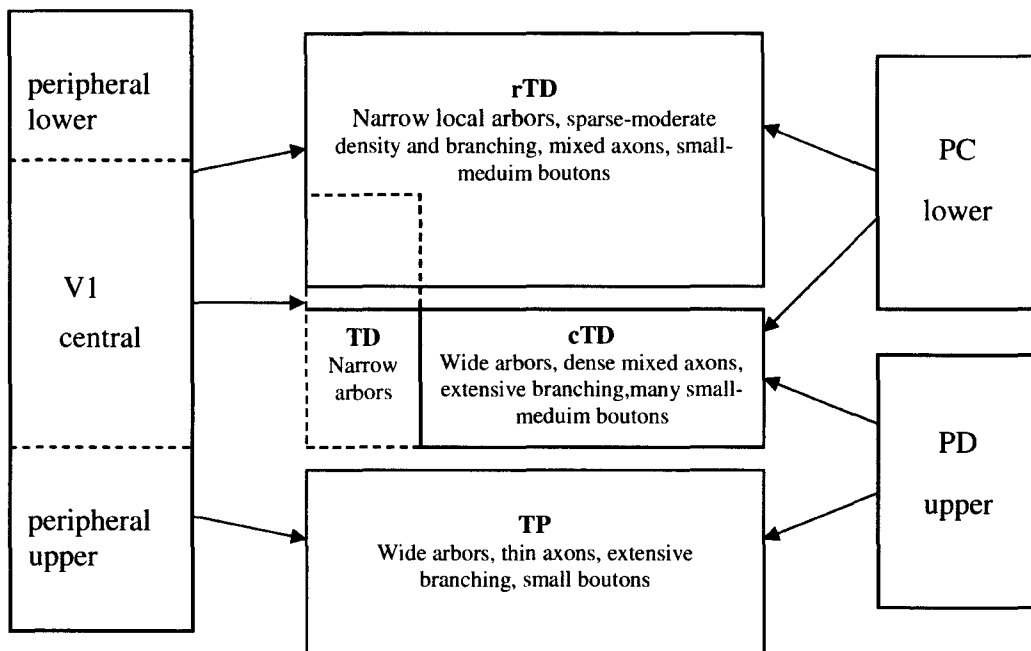
We found three projection "zones" within the TP and TD areas, TP representing the upper peripheral visual field, caudal TD representing the central visual field and

rostral TD representing the lower peripheral visual field. Projections to each of these target zones had different morphological characteristics. This evidence would indicate that there are three functions represented in the combining of these two inputs. As we have discussed, these functions have been found to be related to both motion and attention (Wilke et al. 2010; Snow et al., 2009; Arend et al., 2008; Grieve et al. 2000).

We did not look at the specific neuronal targets of the projections from these two pathways. Additional research could tell us if the V1 projections and the PC and/or PD projections contact the same neuron populations or different neuron populations within TP and TD.

The similarity of axon projections from V1 and the tecto-recipient areas of the pulvinar by target cortical area may indicate that the form of the input from these tecto-pulvinar-cortical pathways (secondary pathways) is virtually indistinguishable from primary visual pathway input. Both primary and secondary pathway information would then combine to impact cortical-pulvinar-cortical loops. This would suggest that attention to visual stimuli shapes perception of visual stimuli as much or more than the original stimulus signal.

Figure 22: Primary and Secondary Pathways Meet in TP and TD



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APPENDIX A:
LIST OF ABBREVIATIONS

Area 17	Primary visual area (V1, striate cortex)
Area 18	Secondary visual area (V2)
Area 19	Tertiary visual area (V3)
ABC	Avidin and biotinylated-horseradish peroxidase
AP	Stereotaxic coordinates anterior to posterior
BDA	Biotinylated dextran amine
cTD	Caudal region of the dorsal temporal area
DAB	Diaminobenzidine
DL	Dorsolateral visual area (V4)
dLGN	Dorsolateral geniculate nucleus
DV	Stereotaxic coordinates dorsal to ventral
CTB	Cholera Toxin B Subunit
hV4	Fourth visual area in humans
ITc	Inferior temporal caudal area
ITi	Inferior temporal intermediate area
ITr	Inferior temporal rostral area
ML	Stereotaxic coordinates medial to latera
MT	Middle temporal area (V5)
Oc1	Occipital area 1
Oc2L	Lateral occipital area 2

Oc2M	Medial occipital area 2
Parv	Parvalbumin
PB	Phosphate buffer
PC	Central pulvinar nucleus
PD	Dorsal pulvinar nucleus
PP	Posterior pulvinar nucleus
PPC	Posterior parietal caudal area
PV	Ventral pulvinar nucleus
rTD	Rostral region of the dorsal temporal area
TA	Temporal anterior area
TAL	Temporal anterior lateral area
TD	Temporal dorsal area
TI	Temporal inferior area
Tm	Temporal medial area
TP	Temporal posterior area
TPI	Temporal posterior inferior area
V1	Primary visual area (area 17, striate cortex)
V2	Secondary visual area (area 18)
V3	Tertiary visual area (area 19)
V4	Fourth visual area (DL)
V5	Fifth visual area (MT)
WFA	Wisteria floribunda lectin

CURRICULUM VITAE

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Personal Information

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Education

University of Louisville, Louisville, KY

M.S. in Anatomical Science and Neurobiology (2009-2012)

Courses towards M.A. Computer Science (1989)

M.A. Prerequisites, Undergraduate (1988)

University of Evansville, Evansville, IN

B.S. in International Business and Economics, Magna Cum Laude (1983)

B.A. in German Language, Magna Cum Laude (1983)

Professional Education/Certifications

University of Louisville

Graduate Teaching Assistant Certification (2011)

Mercury Software

Mercury Winrunner, Mercury Web-Test, Caliber-RM (2000)

Quality Assurance Institute, USA

Certified Software Quality Analyst (1996=1999)

Novell Networks

Novell Network Engineering Classes (1992)

Employment

Farm Credit Services of Mid-America, Louisville, KY (2009)

Quality Analyst

- Programmed Mercury Test Suites for Mortgage Management Software

Accent Marketing, Jeffersonville, IN (2005-2008)

Data, ETL and BI developer

- Programming: Cognos data cubes, SQL, ETL, HTML, VB script, Java script, Business Intelligence (BI)
- Data import, export, integration
- Business requirements, design specifications and use cases
- Support escalation and technical documentation

Ajilon Software Consulting, Louisville, KY (2005)

Quality Assurance Lead, Humana

- Automated test programming for integration testing of pharmacy interface software
- Test case and defect tracking
- Vendor and internal team coordination

Data Programmer, Commonwealth of Kentucky

- Develop prototype database for a grant proposal

- Document requirements and design

The Home Depot, Atlanta, GA (2003-2004)

Quality Assurance Project Lead

- Automated data warehousing testing
- Development and administration of forms, test plans, test scripts and automated tests on the Mercury Test Director Software platform
- Brio reports, Quick Test Pro and SAS. Programming

TEK Systems Software Consulting, Birmingham, AL (2002-2003)

Quality Assurance Project Lead, Southern Progress Media Services

- Establishing the QA processes for the Southern Living at Home development team.
- Leading, training the QA teams
- Software debug and analysis
- Lifecycle design and project documentation

Digital Insight, Atlanta, GA (2000-2002)

Manager of Quality Assurance

- Manage 8-20 member software testing team (variable workload managed with consultants)
- Design and implement requirements-to-release QA processes
- Provide risk-based project management
- Requirements, design and code reviews
- Component to system level testing, metrics and post-project reviews
- Implement Mercury Test Suite. Write Mercury Software based automated tests
- Maintain test platform: XML based messaging server, SQL servers, Microsoft Application Services, and real-time transaction processing

BellSouth Entertainment, Atlanta, GA (1995-1999)

Manager of Quality Assurance

- Manage 5 member integration QA team for digital television and BellSouth.net development involving Unix based video streaming and web hosting, set-top-box hardware and firmware, routers, multiplexers, encoders
- Develop test methodology for hardware and software product development
- Plan and schedule integration testing for BellSouth and 6 vendors
- Participate in design team for computer communications protocol development
- Review vendor designs and code
- Design, develop and manage automated defect tracking system using Remedy software
- Version control, quality improvement

Digital Communications Associates (DCA), Cincinnati, OH (1990-1995)

Manager of Quality Assurance

- Manage a team of 6 software testers, 1 lab technician
- Maintain testing lab hardware/software for all LAN servers on the market with clients on token-ring, Ethernet and StarLAN, and remote access on ATM and Broadband (100+ machines)
- Design and code automated test scripts in C and VB for remote LAN access client/server software

Quality Assurance Analyst

- Test software products providing PC to Unisys mainframe emulation and file transfer

Urban Studies Institute, University of Louisville, Louisville, KY (1988-1990)

Lead Programmer/Analyst II

- Lead a three person team of Programmer / Analysts

- Design, code, test and modify computer programs in COBOL, C, and FORTRAN on an IBM 3390/3380, a VAX/VMS-11 and IBM PCs; used JCL, SAS, SPSS and dBase IV
- Read and debug REXX, and PL/1 programs
- Perform statistical analysis for researchers
- Work with large databases and data files in MVS and CMS
- Network and PC support; troubleshooting for mainframe, PC and LAN users; training classes
- Develop proposal estimates for the computer analysis portion of project bids; maintain and update computer project documentation and quality control procedures; researched new PC software

Wilkerson & Associates Market Research, Louisville, KY (1986-1987)

Market Analyst/Project Manager

- Develop project proposals and direct research projects
- Design survey instruments for telephone, mail and in-person interviewing
- Analyze survey results and write final reports for clients
- Economic and political computer modeling

Kmart Apparel (1983-1986)

Store Manager

- Manage personnel, merchandise and sales for Kmart Apparel store unit within Kmart store facility

Skills

Hardware/Software Implementations

Intel based computer hardware, remote LAN access, routing, bridging, multiplexing, encoding/decoding, modem, data warehousing, database management, data movement (import, export, transform, transfer), internet (b2b, e-commerce, banking), wireless internet, digital TV, wireless digital TV, client-server, real-time transaction processing, terminal emulation, inter-OS file transfer

Programming Languages

SQL, HTML, Visual Basic / VB script, Java script, C, C++, C#, XML, ASP, CGI, Unix shell scripting, Cobol, Fortran, DOS batch

Programming Packages

SAS, SPSS, Matlab, Mercury Suite, Remedy, Cognos, Microsoft ETL, MS Reporting Services, MS Analysis Services, Hyperion Brio, Excel programming language, MS-Access

Programming Paradigms

Structured, object oriented, data driven, OLAP cubing, hypercubing

Servers

Microsoft: IIS, MTS, MSMQ, SQL Server. Other: Novell and misc file servers now defunct

Operating Systems

Windows (3.x to current), DOS, OS/2, HP Unix, AIX, Sun Unix, OS/9 (set-top box), PowerTV, Starsight TV, Palm OS, Unisys, DEC VAX

Topologies

Ethernet, Token Ring, StarLAN, Broadband

Communications Protocols (Networking, Datacom, Telecom, Internet, Wireless)

TCP/IP, UDP/IP, FTP, SNMP, Broadband-modified IP (TCP and UDP), NetBEUI, IPX/SPX, NetBIOS, Named Pipes, XML, Modified XML, DIIS and DIML, HTTP, SSL, ACH, OFX (finance), SLIP, PPP, LCP, NCP, SDLC, HDLC, DSL, ADSL, RF, IR, MMDS, WAP, SMS, Fiber-to-the-Node, ATM, ISDN, X.25, POTS, Async TTY, Bisync Poll Select

Analyzers

LAN Sniffer, FTP's LAN Watch, HP Analyzer, ATM Analyzer, HP Broadband Push Network Decoder, RF Analyzer, Microsoft Performance Analyzer, SNMP capture

Other Analysis and Modeling

Statistical analysis, mathematical modeling, economic and political modeling, neuron modeling

Software management

Product Development Life Cycles (PDLC), IEEE standards, Capability Maturity Modeling (CMM), quality metrics, Cost of Quality, Total Quality Management (TQM), Deming, ISO9000, configuration management, usability analysis, defect management, root cause analysis, Rational Unified Process(RUP) / UML / use cases

Awards

University of Louisville

Graduate Fellowship in Anatomical Science and Neurobiology (2009-2011)

Intercomputer Communications (changed later to Digital Communications Associates)

ICC Excellence in Networking (1992)

ICC Excellence in Terminal Emulation and File Transfer (1991)

Urban Research Institute, University of Louisville

Outstanding Staff Award (1989)

University of Evansville

Kappa Mu Epsilon National Mathematics Honor Society (1981-1983)

Alpha Lamda Delta Freshman Women's Honor Society (1979-1980)

University of Evansville Alumni Scholarship (1979-1983)

Indiana State Scholarship (1979-1983)

Richard E and Oma Meir Scholarship (1979-1983)

Publications

Bonham, G. S., Weir, I. L., Dillihay, D. S., Hunter-Manns, J. and Kentucky Office of Vocational Rehabilitation. (1989). Kentucky statewide study of persons with disabilities. Stage II report. Louisville, KY, Urban Studies Center, College of Urban and Public Affairs, University of Louisville. June 1989 Freq: xix, 89p. app. A 36p., ap.

Interests

Charity work

Hands-on Atlanta, American Lung Association, Habitat for Humanity, Junior Achievement

Hobbies

Painting (oil, acrylic); antiques, vintage books