Quantitative Analysis of the Corticocortical Projections to the Middle Temporal Area in the Marmoset Monkey: Evolutionary and Functional Implications

The connections of the middle temporal area (MT) were investigated in the marmoset, one of the smallest primates. Reflecting the predictions of studies that modeled cortical allometric growth and development, we found that in adult marmosets MT is connected to a more extensive network of cortical areas than in larger primates, including consistent connections with retrosplenial, cingulate, and parahippocampal areas, and more widespread connections with temporal, frontal, and parietal areas. Quantitative analyses reveal that MT receives the majority of its afferents from other motion-sensitive areas in the temporal lobe and from the occipitoparietal transition areas, each of these regions containing approximately 20% of the projecting cells. Projections from the primary visual area (V1) and the second visual area (V2) account for approximately 20% of projecting neurons, whereas "ventral stream" and higher-order association areas form quantitatively minor projections. A relationship exists between the percentage of supragranular layer neurons forming the projections from different areas and their putative hierarchical rank. However, this relationship is clearer for projections from ventral stream areas than it is for projections from dorsal stream or frontal areas. These results provide the first quantitative data on the connections of MT and extend current understanding of the relationship between cortical anatomy and function in evolution.

Keywords: connections, evolution, hierarchical organization, marmoset, visual cortex

Introduction

In this paper, we report on the corticocortical projections to the middle temporal area (MT), a subdivision of the visual cortex common to all primates, which is important for the analysis of motion (Allman and Kaas 1971; Zeki 1974; Van Essen and others 1981; Rosa and Elston 1998). Whereas previous studies have charted the connections of MT (Spatz and Tiggges 1972; Maunsell and Van Essen 1983; Ungerleider and Desimone 1986; Krubitzer and Kaas 1990; Rosa and others 1993), we were motivated by the need to obtain quantitative data regarding the cortical areas and layers that form these projections in order to test hypotheses related to the evolutionary scaling of corticocortical connections and the hierarchical processing of information in the cortex. Marmosets, which are among the smallest anthropoid primates (~350 g adult body weight), were chosen as the model for the present investigation.

It has been suggested that patterns of neuronal connections change systematically as a function of overall brain size and that such changes are likely to have significant functional consequences (e.g., Rilling and Insel 1999; Changizi and Shimojo 2005). Modeling studies have predicted that the patterns of cortical connections vary as a function of number of neurons, with larger brains requiring more segregation between subsystems of areas in comparison with smaller brains in order to maintain a similar level of processing efficiency (Ringo 1991). From a developmental viewpoint, it has also been proposed that areas and nuclei that become relatively enlarged in the course of the evolution of a given species are likely to retain more diverse connections into adulthood by virtue of being advantaged during competitive interactions that shape neural circuitry in early life (Deacon 1990; Striedter 2005). These hypotheses carry specific predictions, which can be addressed by the present study in the marmoset. Both the small size of the brain and the relatively large size of MT in this species, as a fraction of the total area of the neocortex (Rosa and Tweedale 2005), converge to predict that marmoset MT is connected with a more diverse complement of cortical fields, in comparison to that observed in larger species of monkey. Testing whether or not this is really the case was our first objective. In parallel, we also sought to quantify the proportions of cells projecting to MT from different cortical fields. Although to our knowledge this is the first information of this nature available in any primate, it is a first necessary step towards precise comparisons involving the relative strength of different corticocortical connections in different species. A quantitative understanding of the rules according to which the connections between cortical areas change in evolution may in turn highlight features that will help us predict the ways in which the human cortex is likely to differ from that of primate animal models.

Quantitative analyses of connectional patterns are also important in addressing functional questions. Studies in the macaque and cat have suggested that the proportion of labeled cells located in the supragranular layers (%SLN) can yield valuable information on the hierarchical processing rank of visual areas (Barone and others 2000; Grant and Hilgetag 2005). However, to date the patterns of projections to MT have only been described in qualitative terms, and as a result limited inferences can be made with regard to the anatomic-hierarchical organization of the dorsal stream. Thus, our second objective was to quantify the laminar characteristics of the corticocortical projections to MT in order to seek a better understanding of the anatomical-hierarchical structure of the cortical areas responsible for the processing visual information in New World monkeys.

Materials and Methods

Experiments were approved by the Monash University Animal Experimentation Ethics Committee, which also monitored the welfare of the animals. Guidelines of the Australian Code of Practice for the Care and Use of Animals for Scientific Purposes were followed. The present data are based on the analysis of retrograde tracer injections in area MT of 3 adult marmosets. These injections were selected for the present analysis...
from a much larger database (results to be reported separately) on the basis of 3 criteria. First, they employed the same tracers (Diamidino Yellow [DY] and Fast Blue [FB]) that were used in the most recent investigations of the afferent connections of MT in macaque and Cebus monkeys as well as in quantitative studies of laminar patterns of corticocortical connections in the macaque (Ungerleider and Desimone 1986; Rosa and others 1993; Barone and others 2000). Second, there was no encroachment of the white matter by the injection core (Fig. 1). Third, the injections were contained within area MT, as defined by myeloarchitecture (Rosa and Elston 1998). In case CJ56-FB the injection

![Figure 1](image1.png) Figure 1. Laminar extent of the injections of DY (case CJ44) and FB (cases CJ56 and CJ59) that form the basis of the present study, as observed under the fluorescence microscope. These sections represent the deepest level of encroachment of the infragranular layers by the injections. The white dashed line indicates the boundary between layer VI and the white matter. The illustration of the injection site from case CJ44 was photographed from a section that had been counterstained for cytochrome oxidase, hence resulting in some loss of fluorescence; nonetheless, the injection core is clearly delineated. In the panel corresponding to case CJ59, part of a DY injection is visible near the top left corner; this injection was not analyzed as part of the present study. Scale bar = 500 μm.

![Figure 2](image2.png) Figure 2. Summary of the cortical subdivisions of the marmoset considered in the present study. The main panel is a 2-dimensional, “unfolded” view of the marmoset right cerebral cortex, excluding the hippocampal formation. This demonstrates the format used for illustration of individual cases (Figs. 3–5). Due to the relatively high degree of intrinsic curvature of the marmoset cortex, 4 discontinuities were introduced along the perimeter of the map: 1 following the perimeter of V1 (e.g., Van Essen and Maunsell 1980), 1 along the dorsal surface of the frontal lobe, 1 along the frontal pole, and 1 in the rostral portion of the temporal lobe (arrows). Regions shaded in light gray are those normally visible on the surface of the brain from lateral or dorsal perspectives, those shaded medium gray are on the cortical surface but normally hidden on the ventral and midline surfaces of the cortex, and those shaded dark gray are hidden in sulci. Dashed lines indicate the borders of areas or regions, and the asterisk indicates corresponding points on the 2 sides of a discontinuity across the prostriate area, in the rostral part of the calcarine sulcus. Scale bar = 5 mm. Abbreviations: 3a, 3b, 4, 23, 29, 30: cytoarchitectural areas, modified from Brodmann (1909); 19m: medial subdivision of area 19; 23v: ventral subdivision of area 23; 6d, 6m, and 6v: dorsal, medial, and ventral subdivisions of the premotor cortex (Burman and others 2005); A1: primary auditory area; 15/STP: a region that probably includes higher-order auditory areas as well as the superior temporal polysensory area; DA: dorsoanterior area; Di: dorsointermediate area; DM: dorsomedial area; ER: entorhinal area; FST: fundus of superior temporal area; GFC: granular frontal cortex (areas 8, 9, 10, 12/45, and 46 of Burman and others 2005); 1Td: dorsal subdivision of the inferior temporal cortex; 1Tv: ventral subdivision of the inferior temporal cortex; MST: medial superior temporal area; MT: middle temporal area; MTC: middle temporal crescent; PPd: dorsal subdivision of the posterior parietal cortex; PPm: medial subdivision of the posterior parietal cortex; PPv: ventral subdivision of the posterior parietal cortex; PR: perirhinal cortex (area 36); pro: prostriate area; som: nonprimary somatosensory areas; TF and TH: temporal fields F and H (Von Bonin and Bailey 1947); V1: primary visual area; V2: second visual area; VLA: ventrolateral anterior area; VLP: ventrolateral posterior area.
was approximately centered on the representation of the horizontal meridian (Fig. 3), whereas in cases CJ44-DY (Fig. 4) and CJ59-FB (Fig. 5) the location was progressively more ventral (i.e., including the upper quadrant representation to a larger extent). The injection in CJ44 was smaller, and more restricted in terms of inclusion of the infragranular layers, in comparison with the other 2 cases. Nonetheless, as demonstrated in the Results, the observations were highly consistent across cases.

**Surgical Procedures and Retrograde Dye Injections**

The animals were premedicated with intramuscular (IM) injections of diazepam (3.0 mg kg\(^{-1}\)) and atropine (0.2 mg kg\(^{-1}\)) and, after 30 min, were anaesthetized with ketamine (50 mg kg\(^{-1}\)) and xylazine (3 mg kg\(^{-1}\)). They were placed in a stereotaxic frame and the occipital cortex was exposed. The retrograde fluorescent tracers (DY and FB) were directly applied into the cortex as crystals (approximately 200 \(\mu\)m in diameter) with the aid of blunt tungsten wires (Rosa and others 2005). Stereotaxic coordinates obtained in the course of previous studies of marmoset visual cortex (Rosa and Elston 1998) guided the placement of the injections (as explained later, their exact location in relation to cortical layers and areal boundaries was assessed by post-mortem histological reconstruction). The cortex was covered with a section of sterile soft contact lens, the piece of bone removed during the craniotomy was fixed back in place with dental acrylic, and the wound was sutured. Analgesics (oral paracetamol, 3 drops every 6 h) and antibiotics (Norocillin, 0.1 mL) were given routinely for the first 24 h postsurgery. The animals were returned to their home cages for a period of 2 weeks, after which electrophysiological recordings were conducted in a terminal recording session for projects unrelated to the present report (Lui and others 2005a; see Bourne and Rosa 2003, for details of the preparation for recordings).

**Perfusion, Fixation, and Tissue Processing**

At the end of the experiments the animals were deeply anaesthetized with sodium pentobarbitone (100 mg kg\(^{-1}\), IM) and perfused transcardially with 1 L of heparinized saline followed by 1 L of 4% paraformaldehyde in 0.1 M phosphate buffer (pH 7.4). The brains were postfixed in the same medium for a minimum of 1 day before being cryoprotected by immersion in buffered paraformaldehyde solution containing increasing concentrations of sucrose (5%, 10%, and 20%). Frozen 40-\(\mu\)m-thick sections were cut using a cryostat. One series of sections was mounted onto gelatinized slides and air-dried for later staining with cresyl violet, whereas a second series was kept in phosphate buffer and stained free floating either for cytochrome oxidase activity (Wong-Riley 1979) or for neurofilament using antibody SMI-32 (Sternberger LA and Sternberger NH 1983; Bourne and others 2003).
2005). A third series was stored in 10% formalin for at least 2 weeks before staining for myelin, with either the Gallyas (1979) stain or Schmued (1990) gold chloride stain. Finally, a series of sections was mounted unstained on gelatinized slides for the analysis of cells labeled with fluorescent tracers after quick dehydration in 100% ethanol, clearing in xylene, and coverslipping with synthetic resin.

**Documentation of Results and Analysis**

Sections were examined using a Zeiss Axioplan 2 epifluorescence microscope. Labeled neurons were identified using a 20× dry objective. The locations of labeled neurons were mapped with a digitizing system (MD Plot3, Accustage, Shoreview, Minnesota). Two-dimensional reconstructions of the cortical surface were obtained by graphically "unfolding" contours of layer IV of sections 400 μm apart, in such a way as to keep the neighborhood relationships between and within sections (Van Essen and Maunsell 1980). The locations of the injection sites, labeled neurons, and architectural transitions were also projected onto the same maps, allowing correlation between different sets of data. In the present paper, the borders of areas are shown as transition zones of various widths, which reflect various sources of uncertainty arising from methodological factors. These include the separation between sections used in the analysis, the test-retest variability (assessed by repeated plotting by the same observer on different days), the slightly different estimates provided by different architectural methods, and, in some regions, the interference of histological artifacts. Thus, our illustrations distinguish between core regions, which definitely belong to a given architectural field, and fringe zones, which could belong to either of 2 adjacent areas. This allows for a more objective evaluation of the error involved in the interpretation of results. For quantitative analyses, cells within these transition zones were assigned to either of 2 adjacent areas by bisecting these zones into equal halves. Photomicrographs were obtained through Zeiss Pan-Neofluar objectives and acquired as digital images using a Zeiss AxioCam and AxioVision v4.2 software (Carl Zeiss, Oberkochen, Germany).

**Assignment of Label to Cortical Areas, and Basis for Comparisons with Other Species**

A summary map of our current understanding of the cortical areas of New World monkeys is illustrated in Figure 2. Many of the projections to MT charted in the present study originated in areas that have been mapped in detail by previous electrophysiological, anatomical, and architectural studies, including the primary visual area (V1; Fritsches and Rosa 1996), the second visual area (V2; Rosa and others 1997), areas in the dorsomedial cortex rostral to V2 (Rosa and Schmid 1995; Rosa and others 2005), lateral and ventral occipital areas (Rosa and Tweedale 2000), the motion-sensitive cortices surrounding MT (Rosa and others 1993; Rosa and Elston 1998), and the dorsolateral frontal cortex (Burman and others 2005). However, we have also observed labeled
neurons in regions of the occipital, parietal, and temporal lobes that have not yet been characterized in the marmoset. In order to provide both a framework for our data analysis and a basis for comparisons with studies in other species, we have conducted a preliminary architectural study of these regions. The relevant histological distinctions will be illustrated in the Results in parallel with the consideration of the sources of projections to MT.

The charting of primate cortical areas remains subject to an ongoing process of refinement, with definite identities of homologous areas being uncertain in many cases (Rosa and Tweedale, 2004). This results in difficulties for any attempt to compare patterns of connections in different species. Our approach has been one of focusing on broader subdivisions that can be reliably identified by architectural methods rather than dwelling on the detailed areal subdivisions (Table 1). For example, there are strong similarities between the marmoset dorsomedial area (DM) and the densely myelinated fields of the macaque ammunent gyrus and parietooccipital sulcus ("dorsal V3" and the parietooccipital area, PO) to suggest that we are dealing with corresponding regions (Rosa and Tweedale 2001; Lui and others 2005b). Likewise, the questions we intend to address do not hinge on an exact understanding of how many areas exist in the cortical belt rostral to DM; rather, we point out that projections from areas in a corresponding geographical location (V3A, the posterior intraparietal area, and V6A) have also been described in previous studies of the macaque and Cebus monkeys (Ungerfeider and Desimone 1986; Rosa and others 1993; Shipp and others 1998). This regional analysis based on architecture and relative location provided a conservative method for identification of likely homologies, and hence conclusions about which of the connections we observed in the marmoset are either nonexistent (or small enough to have escaped detection with similar methods) in larger monkeys (Table 1). It is possible, however, that our analysis may have underestimated the extent of the differences between species, as the number of cortical areas is likely to increase in proportion to brain size, in some cases through subdivision of preexisting fields (Changizi and Shimojo 2005; Striedter 2005).

**Results**

Figures 3–5 summarize the spatial distribution of projections to MT in graphically “unfolded” reconstructions of the cerebral cortex. Each of the 3 injections resulted in labeled cells covering a large portion of V1 in the calcarine sulcus, corresponding to eccentricities between 5° and the temporal edge of the binocular field of vision (Fritsches and Rosa 1996; see inserts in Figs. 3–5 for estimates). A summary of the quantitative characteristics of the projections to MT are summarized in Table 2. The proportion of corticocortical associations originating in different areas is illustrated in Figure 6, whereas the laminar distribution of MT-projecting neurons is detailed in Figures 7 and 8.

**Figure 5.** Location of labeled neurons after an injection in area MT (case CJ59-FB). Conventions as in the caption of Figure 3.
Projections from Caudal Occipital Areas, V1 and V2

Neurons in V1 and V2 formed major components of the corticocortical projections to MT, corresponding to 13.2% and 7.3% of the total labeled cells, respectively (Fig. 6). Most retrogradely labeled neurons in V1 were located at the level of the striate cortex (i.e., layer IIIc, or “layer IVb” of Brodmann’s nomenclature), with only scattered neurons in the infragranular layers (see also Spatz 1977). Correspondingly, the V1 projection was characterized by the highest relative proportion of supragranular cells (average %SLN = 99.0, Figs. 7A and 8). Whereas labeled infragranular cells were observed more frequently in V2 (Fig. 7B), the projections from this area also showed a strong supragranular bias (average %SLN = 81.3; Fig. 8), which was unique among the extrastriate connections of MT (see also Table 2).

Projections from Putative Dorsal Stream–Associated Areas

Superior Temporal Cortex

Approximately one-third of the total cortical projections to MT originated in surrounding areas, including the middle temporal cortex (MTc), the medial superior temporal area (MST), and the fundus of the superior temporal area (FST). The architectural criteria for definition of these areas have been detailed previously (Ungerleider and Desimone 1986; Rosa and others 1993; Rosa and Elston 1998). Our results indicate that the MTc is the source of the strongest extrinsic cortical projection to MT, harboring 17.0% of all labeled cells. The retrograde label was found covering most of the MTc but was sparser in the posterior part of this area (foveal representation; Figs. 3–5). Although afferents from MST and FST were less numerous (4.7% and 11.3% of the total labeled cells, respectively), they were still quite substantial in comparison with those originating in most other cortical areas (Fig. 6). The projections from the MTc, MST, and FST were characterized by progressively lower average proportions of cells located in the supragranular layers (39.7%, 31.1%, and 14%, respectively; see Figs. 7C–E). However, the

Table 1
Correspondence between cortical fields in marmosets, macaques, and Cebus monkeys and their connections with area MT

<table>
<thead>
<tr>
<th>Marmoset (M)</th>
<th>Macaque (M)</th>
<th>Cebus (C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MTc</td>
<td>V4t; possibly combined with ventral part of FST</td>
<td>V4t; possibly combined with dorsal part of FST</td>
</tr>
<tr>
<td>MST</td>
<td>MTST2</td>
<td>MTST1 + MTST2</td>
</tr>
<tr>
<td></td>
<td>MTSta + MTStdp + MTStm</td>
<td></td>
</tr>
<tr>
<td>FST</td>
<td>FST2,4</td>
<td>FST2,4</td>
</tr>
<tr>
<td>DM</td>
<td>V6d, V6d+</td>
<td>PO + densely myelinated dorsal V3 [V3d]</td>
</tr>
<tr>
<td>Areas rostral to DM (GB + DA + PPm)</td>
<td>V3a + PP + MDP</td>
<td>DX + PP + Pd</td>
</tr>
<tr>
<td></td>
<td>V3a + V6a</td>
<td></td>
</tr>
<tr>
<td>pro</td>
<td>pro</td>
<td>pro</td>
</tr>
<tr>
<td>PPd</td>
<td>LIPv + VIPb</td>
<td>LIPv + VIP</td>
</tr>
<tr>
<td>PPv (includes DOT+)</td>
<td>DP + 7a</td>
<td>DP + 7a</td>
</tr>
<tr>
<td>VLP</td>
<td>V3a + posterior V4d2</td>
<td>V3b16</td>
</tr>
<tr>
<td>VLA</td>
<td>V4v + rostral V4d2</td>
<td>V3 (excluding anterior gyrus)</td>
</tr>
<tr>
<td>ITd</td>
<td>Rostral STS, beyond FST2</td>
<td>Rostral STS beyond FST2</td>
</tr>
<tr>
<td>ITv</td>
<td>TVe</td>
<td>TVe</td>
</tr>
<tr>
<td>GFC (BAv + 12/45 + 46)</td>
<td>FEF-2VA + 12 + 45 + 46p</td>
<td>FEF-3</td>
</tr>
<tr>
<td>6d</td>
<td>60v</td>
<td>6d</td>
</tr>
<tr>
<td>TF</td>
<td>TF</td>
<td>TVp</td>
</tr>
<tr>
<td>TH</td>
<td>TH</td>
<td>TH</td>
</tr>
<tr>
<td>som</td>
<td>S21/1, 27</td>
<td>?</td>
</tr>
<tr>
<td>aud</td>
<td>Tpt, TAAa, STT</td>
<td>?</td>
</tr>
</tbody>
</table>

Table 2
Quantitative characteristics of projections to area MT (data pooled from 3 cases)

<table>
<thead>
<tr>
<th>Area or region</th>
<th>Supragranular cells (n)</th>
<th>Infragranular cells (n)</th>
<th>SLN%</th>
<th>Total labeled cells (%)</th>
</tr>
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<tbody>
<tr>
<td>V1</td>
<td>4907</td>
<td>49</td>
<td>99.0</td>
<td>13.19</td>
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<tr>
<td>V2</td>
<td>2225</td>
<td>512</td>
<td>81.3</td>
<td>7.28</td>
</tr>
<tr>
<td>MTc</td>
<td>2535</td>
<td>3858</td>
<td>39.7</td>
<td>10.01</td>
</tr>
<tr>
<td>MST</td>
<td>549</td>
<td>1217</td>
<td>31.1</td>
<td>4.70</td>
</tr>
<tr>
<td>FST</td>
<td>595</td>
<td>3645</td>
<td>14.0</td>
<td>11.28</td>
</tr>
<tr>
<td>DM</td>
<td>1405</td>
<td>2006</td>
<td>41.2</td>
<td>9.08</td>
</tr>
<tr>
<td>DA/DP/PPm</td>
<td>1465</td>
<td>2716</td>
<td>35.0</td>
<td>11.13</td>
</tr>
<tr>
<td>19m</td>
<td>1200</td>
<td>2266</td>
<td>34.6</td>
<td>9.22</td>
</tr>
<tr>
<td>23v</td>
<td>53</td>
<td>175</td>
<td>23.2</td>
<td>6.61</td>
</tr>
<tr>
<td>23</td>
<td>4</td>
<td>65</td>
<td>5.8</td>
<td>0.18</td>
</tr>
<tr>
<td>pro</td>
<td>26</td>
<td>61</td>
<td>29.9</td>
<td>0.23</td>
</tr>
<tr>
<td>PPd/PPv</td>
<td>444</td>
<td>1867</td>
<td>19.2</td>
<td>6.15</td>
</tr>
<tr>
<td>VLP</td>
<td>358</td>
<td>340</td>
<td>51.3</td>
<td>1.86</td>
</tr>
<tr>
<td>VLA</td>
<td>204</td>
<td>367</td>
<td>35.7</td>
<td>1.52</td>
</tr>
<tr>
<td>ITd</td>
<td>112</td>
<td>717</td>
<td>13.5</td>
<td>2.21</td>
</tr>
<tr>
<td>ITv</td>
<td>0</td>
<td>87</td>
<td>0.0</td>
<td>0.23</td>
</tr>
<tr>
<td>GFC</td>
<td>432</td>
<td>452</td>
<td>48.9</td>
<td>2.35</td>
</tr>
<tr>
<td>6d</td>
<td>54</td>
<td>65</td>
<td>45.4</td>
<td>0.32</td>
</tr>
<tr>
<td>TF</td>
<td>8</td>
<td>439</td>
<td>1.8</td>
<td>0.19</td>
</tr>
<tr>
<td>TH</td>
<td>0</td>
<td>44</td>
<td>0.0</td>
<td>0.12</td>
</tr>
<tr>
<td>som</td>
<td>6</td>
<td>27</td>
<td>18.2</td>
<td>0.09</td>
</tr>
<tr>
<td>aud</td>
<td>7</td>
<td>16</td>
<td>0.4</td>
<td>0.06</td>
</tr>
</tbody>
</table>

*Classification of connections: --, not reported; □, inconsistent or questionable; +, consistent.

Gray shading indicates differences in connections with MT.

Superscript numbers denote references: 1, Present study; 2, Ungerleider and Desimone (1986); 3, Rosa and others (1993); 4, Lewis and Van Essen (2001); 5, Krubitzer and Kaas (1990b); 6, Fiorani and others (1989); 7, Galletti and others (1999); 8, Rosa and Tweedale (2001); 9, Neuenschwander and others (1994); 10, Rosa and Schmid (1995); 11, Rosa and Tweedale (2005); 12, Kobayashi and Amaral (2003); 13, Colby and others (1998); 14, Rosa and Tweedale (2000); 15, Boussaoud and others (1990); 16, Rosa and others (2000); 17, Gattass and others (1998); 18, Filion and others (1998); 19, Burman and others (2005); 20, Gattass and others (2005).
exact %SLN values were somewhat variable between animals (Fig. 8).

**Dorsal Extrastriate Areas**

The densely myelinated fields of the occipitoparietal transition proved to be major sources of projections to MT. Among these is the DM, a topographic representation of the visual field that receives strong direct projections from V1 (Krubitzer and Kaas 1993; Rosa and others 2005). Whereas the cortical belt located rostral to DM is less well understood, there is physiological evidence to suggest the existence of visuotopically organized subdivisions (the putative dorsoanterior [DA] and dorsointermediate [DI] areas; Rosa and Schmid 1995) as well as a nontopographically organized “medial subdivision of the posterior parietal cortex” (PPm; Rosa and Tweedale 2005; see Fig. 2). Our results demonstrate that labeled cells in DM corresponded to 9.1% of the cortical afferents of MT (Fig. 6), with a further 11.1% originating in the belt rostral to DM. Although projections from the lateral part of the rostral belt (putative areas DA and DI) were observed consistently, projections from the medial part (PPm) were noticeably weaker in case CJ59. The proportion of label located in the supragranular layers was higher in DM (%SLN = 41.2; Figs. 7F and 8) than in the areas located rostral to it (%SLN = 35.0; Figs. 7G and 8).

**Medial Extrastriate Cortex**

We observed consistent interconnections between various areas located along the banks of the midline fissure and MT. A major projection (9.2% of all labeled cortical cells) originated in the portion of area 19 interposed between the peripheral representations of areas DM and V2. This region (labeled area 19m in Figs. 2 and 9A,B) probably includes the medial (M) and parietooccipital medial (POm) regions recognized by other authors (Table 1); however, it is unclear whether these are wholly separate areas (Rosa and Schmid 1995). Roughly one-third of the labeled projection neurons in area 19m were in the supragranular layers (%SLN = 34.6; Fig. 7H). As shown in Figures 3–5, projections to MT also originated from the posterior cingulate and retrosplenial regions, including likely homologues of cytoarchitectural areas 23, 23v, and prostriata of other species (Kobayashi and Amaral 2000; Ding and others 2003). The architectural characteristics of these fields in the marmoset are illustrated in Figure 10. Projections from area 23v corresponded to 0.6% of the affrents of MT, whereas those from the more anterior parts of area 23 were even less numerous (15–36 labeled neurons per animal, corresponding to 0.2% of the MT-projecting cells). Finally, small numbers of labeled cells were also found in area prostriata (0.2% of total label). The proportions of supragranular labeled cells in areas 23, 23v, and prostriata were variable between cases, probably reflecting the small samples. Nonetheless, infragranular biases were typically observed (Fig. 8).

**Posterior Parietal Cortex**

The subdivisions of the posterior parietal cortex (areas PE, PF, and PG of Von Bonin and Bailey 1947) are not well understood in New World monkeys. Our analyses of the present material reveal that distinct dorsal and ventral cytoarchitectural subfields (PPd and PPv) can be defined in the posterior parietal region (Fig. 9B) and that most projections to MT originated from the posterolateral part of PPd (Figs. 3–5). Projections from PPv were sparser and originated primarily in the region adjacent to the MTC, which is visuotopically organized (Rosa and Tweedale 2000). Overall, cells in the posterior parietal cortex formed 6.2% of the cortical projections to MT. The proportion of projecting cells located in the supragranular layers was lower in this region than in most other subdivisions of the dorsal stream (average %SLN = 19.2; Figs. 7I and 8). There was no difference in laminar emphasis between projections from PPd and PPv.

**Projections from Putative Ventral Stream–Associated Areas**

**Ventrolateral Areas**

Based on previous electrophysiological mapping and architectural studies (Rosa and Tweedale 2000), we recognize 2...
visuotopically organized areas in the lateral and ventral extrastriate cortices of the marmoset: ventrolateral posterior area (VLP) and ventrolateral anterior area (VLA). The cortex in this region forms strong projections to the inferior temporal cortex (Steele and others 1991; Weller and Steele 1992), indicating that VLP and VLA correspond to early stations of the ventral stream. The present results demonstrate that these areas are only weakly interconnected with MT, with cells in VLP and VLA corresponding to 1.9% and 1.5% of the labeled units, respectively. Although the labeled cells in VLP were nearly equally divided between supragranular and infragranular layers (%SLN = 51.3; Fig. 7J), projections from VLA showed a mild infragranular bias (%SLN = 35.7).

Inferior Temporal Areas

The main projection from the inferior temporal cortex to MT originates in a region anterior and ventral to FST, which has the cytoarchitectural characteristics of temporal field E (TE) of Von Bonin and Bailey (1947). This region, labeled dorsal subdivision of the inferior temporal area (ITd) in Figures 2–5 and 11A, accounted for 2.2% of all cortical projections to MT. Much weaker projections (<50 cells per animal, or 0.2% of the total label) originated from the ventral part of the inferior temporal cortex, which is characterized by less densely populated layers IV and VI, as evidenced in Nissl-stained materials (ventral subdivision of the inferior temporal cortex [ITv]; see Fig. 11A). Projections from ITd to MT showed a marked infragranular bias.

Figure 7. Laminar patterns of projections to MT from various visual areas. Each diagram (A–J) illustrates a coronal section, with the gray box highlighting the region shown at high magnification. The locations of labeled neurons are shown relative to the boundaries of the cortical layers (I–VI). The illustrated patterns exemplify the connections from the following areas: A, V1; B, V2; C, MTc; D, MST; E, FST; F, DM; G, OA; H, 19m; I, PPd; J, VLP. Scale bar (in panel J) = 500 μm.
%SLN = 13.5), whereas every labeled cell in ITv was located in the infragranular layers.

Projections from Association Areas

Granular Frontal and Dorsal Premotor Cortex
Labeled cells were found in the dorsolateral and lateral granular frontal cortex, including the putative frontal eye field (cytoarchitectural field 8Av) and adjacent areas (cytoarchitectural fields 12/45, and 46; see Burman and others 2005, for histological criteria). Projections from these fields corresponded to 2.4% of the neurons labeled by MT injections (Fig. 6). In addition, a small projection (0.3% of the MT afferents) originated from the ventral-most portion of the dorsal premotor area (6d). The frontal lobe projections to MT were characterized by near-balanced proportions of labeled cells located in the supragranular and infragranular layers (%SLN = 48.9 for granular frontal areas, %SLN = 45.4 for area 6d).

Parahippocampal Cortex
Labeled neurons were observed in the posteromedial portion of the temporal lobe, in architectural fields TF and TH (temporal fields F and H) (Von Bonin and Bailey 1947; see Fig. 11B). Projections from TF formed 1.2% of the cortical afferents of MT, whereas those from TH were much weaker (1–22 cells per animal, or 0.1% of the labeled neurons). The overwhelming majority of the label in both of these areas was located in infragranular layers (Fig. 8, Table 2).

Putative Polymodal Connections
Scattered cells were observed in locations consistent with the existence of nonvisual afferents to MT. In 2 animals (CJ56 and CJ44; Figs. 3 and 4), small patches of label (5–15 cells) were observed in a location consistent with the second somatosensory area (Krubitzer and Kaas 1990a), although this label may have extended far enough dorsally and caudally to encompass parts of somatosensory areas 1 and 5 (Padberg and others 2005). Labeled neurons were also found in the cortex ventral to the densely myelinated subdivision of the auditory cortex. The labeled region may correspond to parts of the auditory parabelt or the superior temporal polysensory area. These cells were more numerous in cases CJ56 and CJ59 (8 and 13 neurons, respectively, Figs. 3 and 5).

Discussion

Comparison with Larger Primates
Here we report on the pattern of cortical projections to area MT in the marmoset, one of the smallest primates. Whereas earlier studies have described the regional distribution of MT afferents and efferents in various species, it has been only recently that a firm understanding of the boundaries of cortical areas in New World monkeys has been achieved. Thus, although it is accepted that the pattern of cortical projections to MT is generally similar in different primates (Krubitzer and Kaas 1990b), the exact extent of species differences has remained unknown. Reflecting our aim of comparing our results with those obtained in previous studies in larger primates, we have tried to minimize some of the likely sources of error by adopting techniques for histology and reconstruction of the data that are the same as those used by Maunsell and Van Essen (1983), Ungerleider and Desimone (1986) and Rosa and others (1993). However, given that the surface area of MT in the marmoset is only one-fifth of those in the macaque or Cebus (13 mm² vs. 60–70 mm²; Desimone and Ungerleider 1986; Fiorani and others 1989; Rosa and Elston 1998), injections of similar size typically encompass a much larger fraction of the visuotopic map in the former species. Hence, rather than comparing the results of individual cases, it is more appropriate to consider composite patterns of connections (i.e., those revealed by multiple tracer injections) as illustrated in Figure 12. These composite maps highlight basic similarities in the regional distribution of
MT-projecting cells as well as several specific features that support the idea of species differences. For example, the MT-projecting fields in the ventral extrastriate cortex are more widespread in the marmoset, where they extend to the neighborhood of the entorhinal cortex. Moreover, projections that are described as absent or inconsistent in previous studies (e.g., those originating from areas TE and TF) appear to be far more substantial in the marmoset. Other differences include the extent of the frontal, parietal, and medial extrastriate fields that send projections to MT. Indeed, the most obvious impression conveyed by Figure 12 is that a larger fraction of the cerebral cortex is engaged in connections with MT in small monkeys, as compared with larger monkeys. The results obtained by Krubitzer and Kaas (1990b) in 2 marmosets seem to agree, in broad regional terms, with the pattern of MT connection summarized in Figure 12, despite the different criteria for definition of areas in flat-mounted preparations.

Implications for Understanding the Evolution and Development of the Visual Cortex

The brain of the marmoset is approximately 12 times smaller than the macaque brain, which in turn is 15 times smaller than the human brain (Stephan and others 1981). Thus, while acknowledging the likely existence of a similar neural architecture common to all anthropoids, it is also important to investigate how such massive changes in the number of neurons may have impacted on cortical processing. Evolutionary changes in the size of the neocortex are not based on an isometric scaling of the different areas; the primary sensory fields comprise a much larger fraction of the cortex in small primates than in larger primates, whereas the association fields...
of the frontal, temporal, and parietal cortices become relatively larger in the larger species (Deacon 1990; Rosa and Tweedale 2005). These trends seem to correlate with the temporal profile of the development of different brain regions (Finlay and Darlington 1995; Rosa 2002). Being one of the earliest-maturing cortical areas (Bourne and Rosa 2005), MT is “proportionately” much larger (i.e., as a fraction of the total area of the cerebral cortex) in small primates, such as the marmoset, than in macaques and Cebus monkeys (Frahm and others 1998; Rosa and Tweedale 2005; see Table 3).

Previously, we have noted that frontal projections to MT and other visual areas of the marmoset originate not only from the frontal eye field (cytoarchitectural areas 8A and 45 of Walker 1940) but also from adjacent areas 12 and 46 (Burman and others 2005), a result that contrasts with previous observations in larger diurnal monkey species (Ungerleider and Desimone 1986; Boussaoud and others 1990; Rosa and others 1993). The present results further demonstrate that marmoset MT receives several corticocortical projections not described in larger monkeys, supporting the view that the corticocortical connections of a homologous area can vary between primate species. The differences we observed between the marmoset, macaque, and Cebus are consistent with those predicted by evolutionary/developmental theories that link variations in neural connectivity to changes in the relative size of neural structures (Deacon 1990) or to changes in the total size of the neocortex (Ringo 1991). Although these models have been supported by comparative analyses of various other vertebrate model systems (e.g., Nudo and Masterton 1990; Rilling and Insell 1999; Ramnani and others 2005), their validity as a predictor of

Figure 10. Myeloarchitectural (A) and cytoarchitectural (B) characteristics of areas in the rostral portion of the calcarine sulcus and retrosplenial cortex. This coronal section cuts through the representation of the monocular periphery in V1. At this level, V2 is observed only in the ventral bank of the calcarine, separated from V1 by a narrow region of lightly myelinated cortex (area prostriata), which also has a narrow and less densely stained layer IV. Area prostriata is also visible adjacent to V1 on the dorsal bank. The transition between prostriata and area 23v is marked by an increase in the thickness of layer IV and a change in the pattern of myelination. Scale bar = 1 mm.

Figure 11. Cytoarchitectural characteristics of fields in the inferior and medial temporal cortices. (A) Rostral section, showing the transition between the dorsal and ventral subdivisions of the inferior temporal cortex (field TE of Von Bonin and Bailey 1947). Subsector ITd is characterized by a more sharply defined layer IV and denser layer VI in comparison with ITv. Portions of the perirhinal (PR; areas 35 and 36) and entorhinal (ER) cortices are visible in the temporal cortex medial to ITv. (B) Caudal section, showing the transitions between the caudal portion of ITd and fields TF and TH (Von Bonin and Bailey 1947). TF and TH show a thinner layer IV and darkly stained layer VI in comparison with both ITd and ITv. Scale bar = 1 mm.
Allometric changes in corticocortical connectivity has remained largely untested (however, see Changizi and Shimojo 2005). Because the present results constitute the first quantitative assessment of the cortical projections to area MT, there is little basis for comparing our observations in the marmoset with those in other species, other than noting the general emphasis in connections with V1, V2, and the dorsal stream areas. However, our results carry clear predictions in the context of the hypothesis that relates the relative size of cortical areas and the formation of their connections (Deacon 1990). We have previously demonstrated that the homologues of V2, V3 (VLP), and V4 (VLA) are proportionately much larger in either Macaca or Cebus than in the marmoset, whereas the ratio of the surface areas of V1 and MT remains approximately the same in these species (Rosa 2002; Rosa and Tweedale 2005). Thus, one would expect future quantitative studies in the macaque to reveal comparatively larger fractions of the projections to MT originating in areas such as V2 and V4, perhaps at the expense of a relative de-emphasis of the direct connection with V1. These trends would most likely become even more exaggerated in the human brain, where the cortex between V1 and MT expands markedly and is likely to contain evolutionarily “new” areas (Rosa 2002; Dougherty and others 2003; Orban and others 2004; Sereno and Tootell 2005). Such changes in connectional emphasis could in turn have functional implications related to the different mix of synaptic influences on adult MT in different species. In this sense, investigating evolutionary/developmental influences on the adult phenotype may have a wider impact for understanding changes in cortical physiology (Rilling and Insel 1999; Changizi 2001).

### Table 3

<table>
<thead>
<tr>
<th>Species</th>
<th>Area of MT (mm²)</th>
<th>Total area of cortex (mm²)</th>
<th>Fraction MT/total cortex (%)</th>
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</thead>
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<tr>
<td>Callithrix jacchus</td>
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<td>1.142</td>
<td>1.14</td>
</tr>
<tr>
<td>Cebus apella</td>
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<td>14.736</td>
<td>0.46</td>
</tr>
<tr>
<td>Macaca fascicularis</td>
<td>74</td>
<td>14.056</td>
<td>0.53</td>
</tr>
</tbody>
</table>

this being a "lateral" connection, suggesting that VLP and MT studies of MT connections in macaques and heteromodal connections recently reported in other primates (Krubitzer and Kaas 1990b). These results add to the evidence of horseradish peroxidase in MT of marmosets (Figs. 20 and 21 of also been illustrated after 2 injections of wheat germ agglutinin--labeled neurons in corresponding geographical locations have have direct projections to MT in the marmoset. Small patches of suggesting that high-order somatosensory and auditory cortices Our results demonstrate projections from areas in the ventral part of the anterior parietal cortex and superior temporal gyrus, suggesting that high-order somatosensory and auditory cortices have direct projections to MT in the marmoset. Small patches of labeled neurons in corresponding geographical locations have also been illustrated after 2 injections of wheat germ agglutinin--horseradish peroxidase in MT of marmosets (Figs. 20 and 21 of Krubitzer and Kaas 1990b). These results add to the evidence of heteromodal connections recently reported in other primates (Falchier and others 2002; Rockland and Ojima 2003). Previous studies of MT connections in macaques and Cebus monkeys have not reported heteromodal associations. However, rostral portions of the dorsal bank of the superior temporal sulcus, including putative auditory association or polysensory areas, have been shown to project to both MST and FST (Boussaoud and others 1990; Rosa and others 1993). One interpretation of these data links the likely smaller number of areas and neurons in the marmoset cortex (Changizi and Shimojo 2005) and hence fewer processing stages, with the existence of "shortcuts" in sensory-motor analysis and integration (Burman and others 2005).

**Putative Polymodal Associations**

Our results demonstrate projections from areas in the ventral part of the anterior parietal cortex and superior temporal gyrus, suggesting that high-order somatosensory and auditory cortices have direct projections to MT in the marmoset. Small patches of labeled neurons in corresponding geographical locations have also been illustrated after 2 injections of wheat germ agglutinin--horseradish peroxidase in MT of marmosets (Figs. 20 and 21 of Krubitzer and Kaas 1990b). These results add to the evidence of heteromodal connections recently reported in other primates (Falchier and others 2002; Rockland and Ojima 2003). Previous studies of MT connections in macaques and Cebus monkeys have not reported heteromodal associations. However, rostral portions of the dorsal bank of the superior temporal sulcus, including putative auditory association or polysensory areas, have been shown to project to both MST and FST (Boussaoud and others 1990; Rosa and others 1993). One interpretation of these data links the likely smaller number of areas and neurons in the marmoset cortex (Changizi and Shimojo 2005) and hence fewer processing stages, with the existence of "shortcuts" in sensory-motor analysis and integration (Burman and others 2005).

**Implications for Understanding Hierarchical Processing in the Primate Cortex**

A clear association between presumed hierarchical ranking and percentage of labeled cells in the supragranular layers is demonstrated for the projections from ventral stream areas to MT (Fig. 13). The proportion of supragranular cells forming the projection from VLP to MT (%SLN = 51.3) is compatible with this being a "lateral" connection, suggesting that VLP and MT represent equivalent hierarchical stages of processing in the ventral and dorsal streams, respectively. The projection from VLA (%SLN = 35.7) falls near the limit of the ranges characterizing "lateral" and "feedback" connections according to the criteria reported by Grant and Hilgetag (2005); hence, this area may occupy a hierarchical level just above that of MT. Whereas the ventral portions (upper quadrant representations) of VLP and VLA correspond precisely to the ventral portions of V3 and V4 defined in the macaque and Cebus, it is likely that the lower quadrant representations of these areas have both been included in "dorsal V4" of other nomenclatures (Table 1; for discussion see Rosa and Tweedale 2000; Rosa and Manger 2005). Thus, although exact comparisons are impossible, our observations can be related to some extent to those of studies in macaques and Cebus monkeys, which suggested that the projections from V4 to MT include intermixed patches of "lateral" and "feedback" connections in a variable manner (Maunsell and Van Essen 1983; Ungerleider and Desimone 1986; Rosa and others 1993). Most of the previously uncharted projections we observed from higher-order association centers to MT (e.g., those from ITd, ITv, TF, TH, retrosplenial, and posterior cingulate areas) are characterized by strong infragranular predominance, suggesting a clear "feedback" character regardless of whether they are associated with the ventral or the dorsal stream. The clear outliers in this respect were the projections from the granular frontal cortex and area 6d, which appeared to follow a "lateral" pattern.

Our data also reveal a far less obvious correlation between supragranular predominance, and putative rank exists among the projections from dorsal stream areas to MT (Fig. 13). If one adopts the quantitative criteria suggested by Grant and Hilgetag (2005) to interpret our data, MT appears to form "lateral" connections with the MTc and DM, whereas its connections with areas rostral to DM (%SLN = 35) and 19m (%SLN = 34.6) fall near the limit of what may be classified either as "lateral" or "feedback" on the basis of retrograde tracing alone (Fig. 13). In addition, the projection from MST (%SLN = 31.1) suggests that this area is located just above MT in the anatomical hierarchy of the cortex. Similarly, Ungerleider and Desimone (1986) noted, on the basis of both retrograde and anterograde patterns in the macaque, the existence of a number of putative "lateral" connections between MT and dorsal stream areas, including adjacent regions of the superior temporal sulcus, and areas of the annectent gyrus. In our data the only clear "feedback" connections to MT from dorsal stream areas seem to originate in the posterior parietal cortices and FST. The latter area also seems to be involved in the ventral stream, perhaps contributing to shape processing on the basis of motion parallax cues (Boussaoud and others 1990; Rosa and Elston 1998).

The suggestion of different anatomical–hierarchical structures for the dorsal and ventral streams has interesting parallels in other physiological, cytological, and developmental features of the primate visual cortex. Although the response latency grows gradually and predictably between ventral stream areas, dorsal stream areas tend to show far less marked differences with regard to this response parameter (Schmolesky and others 1998). For example, in the macaque, visual responses occur nearly simultaneously in MT, MST, the frontal eye field, and the densely myelinated dorsal cortex rostral to V2 (dorsal V3, see Table 1). These observations suggest a less linear chain of hierarchical processing in the dorsal stream, in comparison with that in the ventral stream (see also Bullier 2001). Investigations
of pyramidal cell morphology in ventral stream areas have revealed a consistent trend for neurons to become larger, more branched, and more spiny in progressively "higher" stations, an observation which contrasts with comparatively mild and less predictable variations among dorsal stream areas (Elston and Rosa 1997, 1998). Finally, the morphological maturation of cortical neurons in early postnatal life follows a clear, gradual developmental sequence along the ventral stream, from V1 to the inferior temporal cortex (Conde and others 1996). In contrast, with the exception of the early development of MT, a pattern of sequential development is much harder to discern in the dorsal stream, where most areas mature quite rapidly (Bourne and Rosa 2005). One possible confounding factor in this regard may be the existence of various parallel pathways within the dorsal stream, such as the proposed "dorsomedial route" through DM and the "dorsolateral route" through MT, formed by areas with contrasting response properties, particularly with respect to widefield motion versus object motion (Rosa and Tweedale 2001; Lui and others 2005b). Whereas the exact manner by which anatomical and functional measures of hierarchical processing relate to each other remains elusive, our data add to the evidence that suggests that dorsal stream areas are characterized by a less strict hierarchical organization, in comparison to ventral stream areas.

**Notes**

Address correspondence to Dr Marcello G. P. Rosa, Department of Physiology, Monash University, Victoria 3800, Australia. Email: Marcello.Rosa@med.monash.edu.au.

**References**


