CONNECTIONS OF THE SUPERIOR COLLICULUS WITH VISUAL BRAIN STRUCTURES IN GALAGOS, TREE SHREWS, AND GRAY SQUIRRELS

By

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To my parents and Adam for all of their support and patience

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CHAPTER 1

INTRODUCTION

The present report is part of a larger effort to understand the evolution of the human, with these particular studies addressing questions about the evolution of subcortical and cortical visual structures in early primates and their close mammalian relatives. To do this, we have used a comparative approach. Comparative studies of brain organization across the major lines of the mammalian radiation can help provide information on not just the evolution of the visual system (Rosa and Krubitzer, 1999), but also on organizational principles, physiological and anatomical characteristics, or behavioral properties underlying visual functions in all mammals as well as characteristics that are unique to a particular species or group of species (Kaas, 1996; Kaas and Preuss, 2003). Closely related species are expected to share more common brain features than more distantly related species (Kaas, 2002, 2005, 2012). By comparing brain features of extant mammals within and across the major lines of the mammalian radiation (Fig. 1.1), we can gain insights about commonalities among mammals that reflect ancestral features as well as differences or specializations that have evolved independently (Kaas, 1987; Northcutt and Kaas, 1995; Preuss et al., 1999; Kaas, 2002; Kaas and Preuss, 2003; Krubitzer et al, 2011; Kaas, 2012). These insights can further provide clues as to which features are more likely to be necessary for a particular function, and which may be unimportant.

Characteristics that are found to be common in all or most members of a group or clade of related species are considered to be inherited from a common ancestor and homologous, whereas traits that are inconsistently distributed within a clade are more likely to have evolved independently and undergone convergent evolution. The

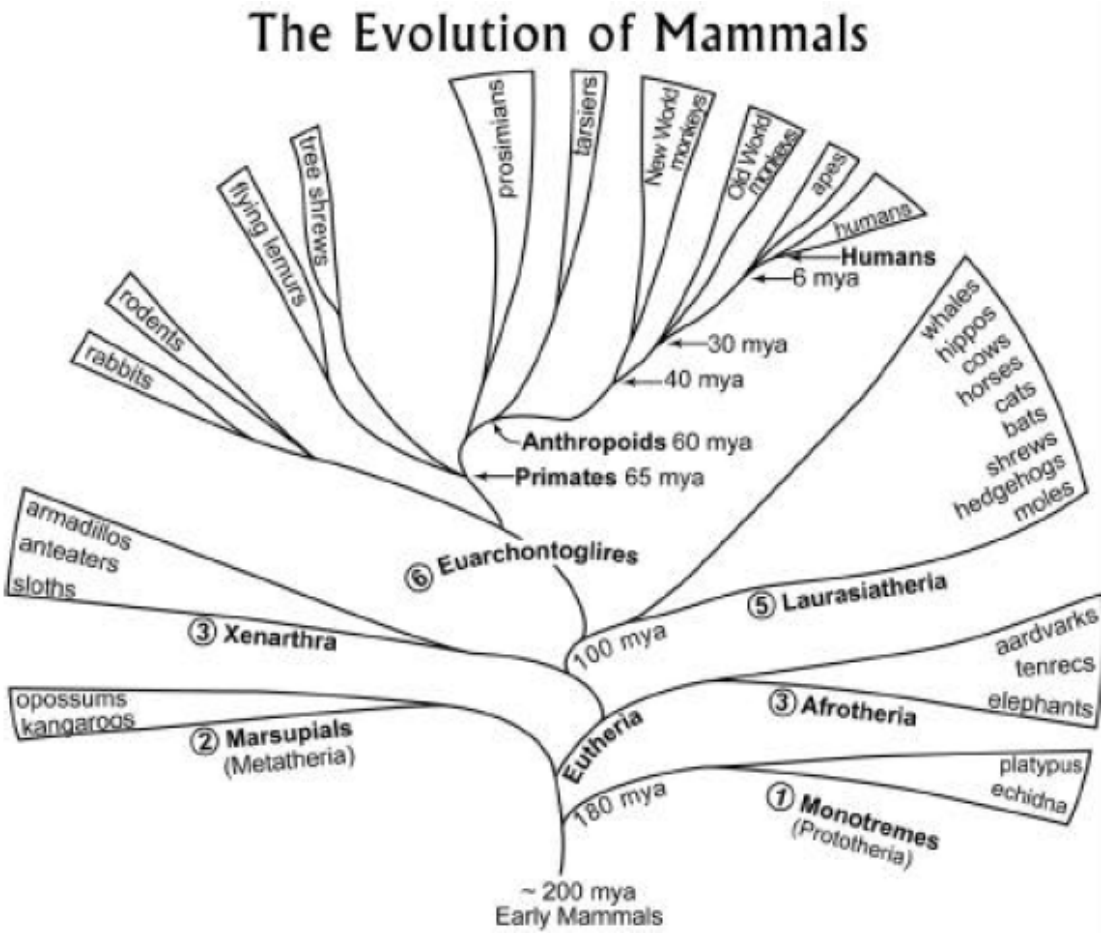

Figure 1.1 The phylogenetic tree of the mammalian radiation. From Kaas, 2005.

concept of convergent evolution is important when considering comparative studies as some traits could have evolved independently across mammalian groups, such as independent modifications in brain structures associated with a diurnal lifestyle or living in an arboreal vs. ground dwelling environment (Campi and Krubitzer, 2010; Krubitzer et

al., 2011). Some examples include the presence of occular dominance columns, or orientation pinwheels in cats and primates (Kaas, 2005; 2013). Understanding the presence and distribution of convergent features can still provide useful clues for determining if and how brain structures have been modified, and whether such modifications take similar forms despite their independent evolution. On the other hand, it is useful to study mammals with similar behavioral and lifestyle characteristics across orders or clades, as mammals with dissimilar characteristics likely have adapted brain features that are significantly divergent (Kaas 1987; Catania et al., 1999; Campi and Krubitzer, 2010; Krubitzer et al., 2011) making homologous structures more difficult to recognize. For instance, studying the visual system in nocturnal rodents, which use their whiskers to navigate their environments, as a model for studying the primate visual system may not be as useful as studying other highly visual rodents (Van Hooser and Nelson, 2006; Manger et al., 2008).

In this report, a focus is directed towards understanding modifications within the Euarchontoglires clade, which branched off from the rest of the mammalian radiation approximately 80 to 100 million years ago (Murphy et al, 2001; Kaas, 2005; Meredith et al., 2011), and comprises of rodents, lagomorphs, flying lemurs, tree shrews and primates, including humans (Fig. 1.1). The ecological niches, diets, and diel patterns of members of the Euarchontoglires clade range substantially. Consequently, members of the Euarchontoglires clade exhibit a wide range of behavioral characteristics that are specifically adapted to their particular environments. As mentioned, such differences in behaviors are often reflected by alterations of the brain structures that play a crucial role in generating these behaviors (Krubitzer et al., 1995; Catania, 2011). Therefore, species

with similar environmental niches likely share similar characteristics in brain organization patterns either by maintaining such characteristics from a common ancestor, or by attaining them through independent convergent modifications.

In order to account for these considerations, the current report focuses on studying the visual system of three highly visual arboreal mammals: prosimian galagos (*Otolemur garnettii*), tree shrews (*Tupaia glis*), and gray squirrels (*Sciurus Carolinensis*), each from a different branch of the Euarchontoglires clade. The main goal is to examine similarities and differences across these three species, not only to discover brain features of the visual system that are likely passed down from a common ancestor or similar ecological niches, but also to explore specializations that each species may have otherwise evolved. What follows is a more in-depth description of why gray squirrels, tree shrews, and galagos were chosen for the current study, along with the reasoning for why studying connections of the superior colliculus is useful for assessing organizational patterns of the visual system.

1.1. SPECIES JUSTIFICATION

Gray squirrels, tree shrews, and galagos are all mammalian species representing different orders of the phylogenetic tree, Rodentia, Scandentia, and Prosimians. Yet, all three species are members of the Euarchontoglires clade. Therefore, they likely have many similar brain characteristics that are common to all Euarchontoglires species, but also have specializations associated with evolutionary divergences in morphology and behavior. Yet, such specializations may not be as disparate as what may be observed when comparing members of different clades, such as in comparing characteristics between primates and cats, which belong the Laurasiatheria clade (Fig. 1.1).

1.1.1. Gray squirrels (Sciurus carolinenesis)

The gray squirrel (Fig. 1.2) is a widely available rodent with an expanded cortex and a well-developed visual system (Van Hooser and Nelson, 2006), which includes large eyes with good optics (Gur and Sivak, 1979; McCourt and Jacobs, 1984), cone dominated retina (West and Dowling, 1975), dichromatic color vision (Blackslee et al., 1988), a large striate cortex (Van Hooser et al., 2003), several extrastriate cortical areas (Hall et al, 1971; Kaas et al., 1989; Wong et al., 2008; Wong and Kaas, 2008), a laminated lateral geniculate nucleus (Kaas et al., 1972), a large and complex pulvinar (Robson and Hall, 1977; Baldwin et al., 2011), and a superior colliculus that is among the largest of all studied mammals (Lane et al., 1971) (Fig. 1.5). But, the most important characteristic of squirrels for the current research is that they are diurnal rodents that use their visual sensory system to navigate their environments, forage for food, and avoid predators (Fitch, 1948; Shorten, 1954; Duncan and Jenkins, 1998) over their somatosensory

system, which is in contrast to other well-studied rodents such as rats and mice. Thus, because of their behavioral similarities with primates, by using vision over other sensory modalities, gray squirrels can serve as an excellent model for bridging the gap between our understanding of visual cortical organization in primates and rodents.

1.1.2. Tree Shrews

Like gray squirrels, tree shrews (Fig. 1.3) are diurnal arboreal mammals, and are one of the closest relatives to primates (Murphy et la., 2001; Meredith et al., 2012). Tree shrews have a well-developed visual system with high visual acuity (Petry et al., 1984), a cone dominated retina (96% cones :Immel and Fisher, 1985), a large striate cortex with several extrastriate visual areas (Kaas et al., 1972; Lyon et al., 1998; Wong and Kaas, 2009), a laminated lateral geniculate nucleus (Conway and Schiller, 1983; Kaas, 2002), a complex pulvinar (Lyon et al., 2003a; Lyon et al., 2003b); and a large and distinctively laminated superior colliculus (Abplanalp, 1970; Lane et al., 1971; Kaas and Huerta, 1988; Kaas, 2002) (See Fig. 1.5). Additionally, tree shrews share many morphological and behavioral characteristics as gray squirrels and were initially considered squirrels when discovered (Kaas, 2002). As tree shrews and squirrels share similarities in body shape, ecological niche, and an emphasis on vision, it seems likely they would share many similar brain features as well.

1.1.3. Galagos

Galagos (Fig. 1.4) are highly visual arboreal mammals. Though they are nocturnal, they have large eyes and well-developed visual structures, such as large and well-laminated lateral geniculate nucleus, a large striate cortex, with expanded visual and visuomotor cortical areas that are also present in anthropoid primates (Wong and Kaas,

2010), and a well-laminated superior colliculus. As such, galagos have been a popular model for studying the visual system (Allman et al., 1973; Wall et al., 1982; Rosa et al., 1997; Collins et al., 2001; Lyon and Kaas, 2002; Xu et al., 2005; Kaskan and Kaas, 2007).

Galagos, being prosimian primates, are thought to be the least derived species within the primate lineage, maintaining many of the cortical organization patterns that may have been present in the early ancestors of primates (Preuss and Goldman-Rakic, 1991a; Kaas, 2007, 2008). As such, the less complex brain of galagos can serve as a good model for comparing brain organization characteristics with non-primate species such as those of squirrels and tree shrews. On the other hand, though galagos share many cortical and subcortical brain features with those of anthropoid primates (Collins et al., 2001; Kaskan and Kaas, 2007), anthropoids have additional features not present in prosimians (Allman et al., 1979; Preuss and Goldman-Rakic, 1991a, b). Therefore, by comparing the brain organization of galagos to those of anthropoid primates, insights can be gained into understanding specializations of brain features that are products of anthropoid evolution.

1.2. WHY STUDY THE SUPERIOR COLLICULUS?

The superior colliculus (also known as the tectum) is an evolutionarily ancient sensorimotor structure located in the midbrain. It was present in the earliest vertebrates (Gaither and Stein, 1979; Stein, 1981; May, 2006; Maximino, 2008), and is found in all present-day mammals (Wurtz and Albano, 1980; Stein, 1981; Dean et al., 1989; May, 2006). As such, the superior colliculus lends itself well for comparative studies. Though the superior colliculus is involved in multiple sensory modalities, the present report will focus on the superior colliculus's role in vision, for which the superior colliculus has two main functions. One is participating in transferring incoming retinal inputs to other subcortical visual structures such as the pulvinar (Kaas and Huerta, 1988; May, 2006). The other main role of the superior colliculus is integrating visual, auditory, and somatosensory information to guide orientation movements of the eyes and head (Casagrande et al., 1972; Harting et al., 1973; Stein et al., 1976; McPeek and Keller, 2004) and possibly limbs (Werner et al., 1997).

One of the hallmarks of the superior colliculus is its laminar structure, which is evident in all studied mammals (Kaas and Huerta, 1988; May, 2006) (Fig. 1.5). The laminar structure of the superior colliculus is highly organized and contains seven main layers including the stratum zonale (SZ), stratum griseum superficiale (SGS), stratum opticum (SO), stratum griseum intermediale (SGI), stratum album intermediale (SAI), stratum griseum profundum (SGP), and the stratum album profundum (SAP) (Fig. 1.5). The superficial layers, consisting of the SZ, SGS, and SO, are almost exclusively responsive to visual stimuli, while intermediate and deep layers are involved in multisensory and motor functions (for review see Kaas and Huerta 1988; May 2006).

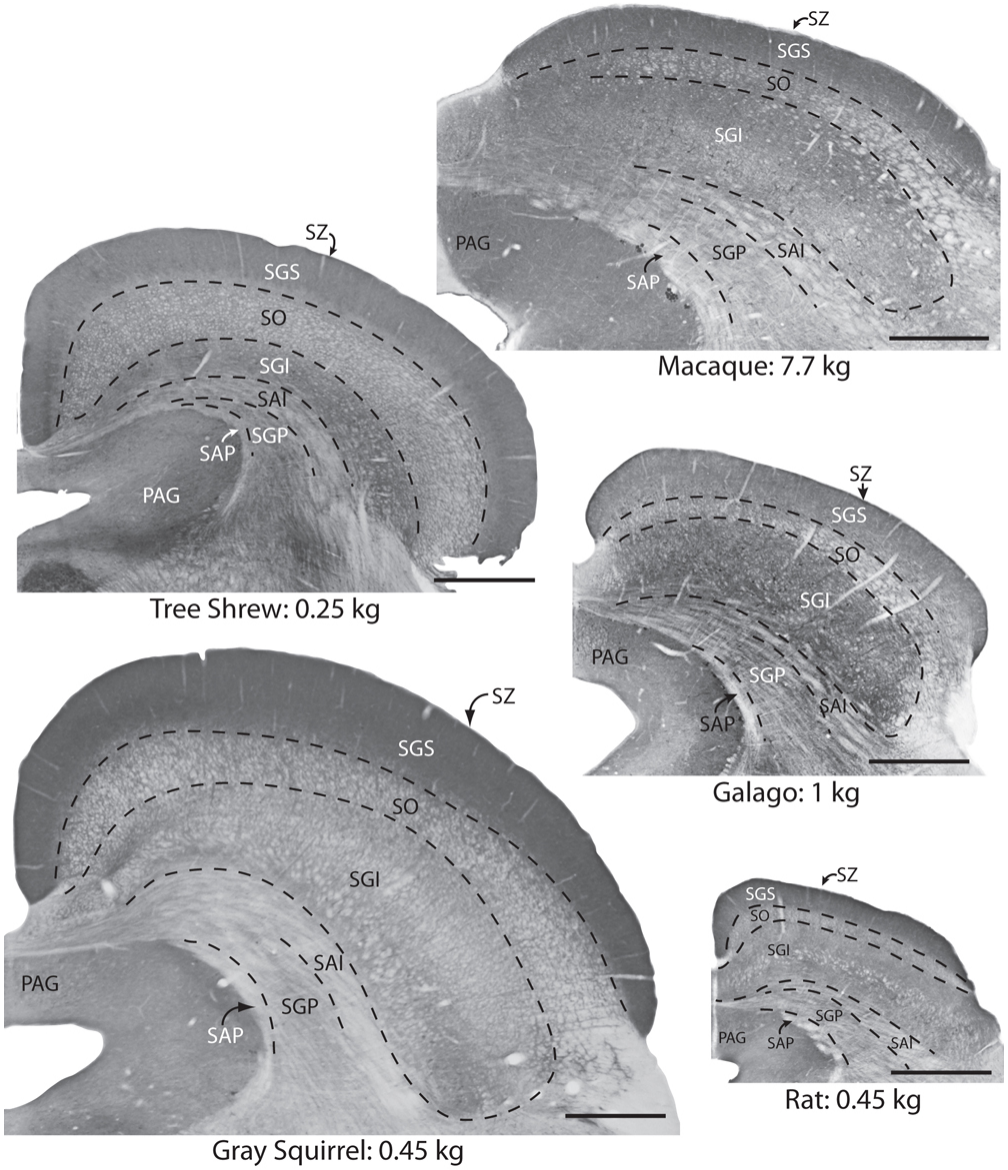

Figure. 1.2. The laminar organization of the superior colliculus across different species within the Euarchontoglires clade. These are coronal sections taken approximately midway through the superior colliculus and processed for cytochrome oxidase. All scale bars are 1mm, and approximate mean weight for each species is given.

When examining the superior colliculus across different mammals, it is hard to not notice the variability in size and laminar structure, not just between species of the same clade but even within the same family such as squirrels and rats (Fig. 1.5). Some influences of the structural variances across the superior colliculus of different mammals

are likely attributable to changes in the number and type of cortical areas across different members of the mammalian radiation (Kaas et al., 2000, 2005), as the superior colliculus receives a great number of cortical inputs (See Kaas and Huerta, 1988; May, 2006 for reviews). Additionally, such variances in the superior colliculus structure could also be attributed to differences in subcortical inputs. For instance, only 10% of the retinal ganglion cells in macaque monkeys project to the superior colliculus (Perry and Cowey, 1984), while in rodents such as mice this number is closer to 70% (Hofbauer and Drager, 1985).

Not only is the superior colliculus organized in a laminar pattern, but it is also has a well-organized topographic map of the visual field. The topographic organization of the superior colliculus has been well studied in gray squirrels (Lane et al., 1971), tree shrews (Lane et al., 1971), and galagos (Lane et al., 1973) (Fig. 1.6). In these and other mammals, the lower visual field is represented laterally and the upper visual field is represented medially. The representation of central vision is located rostrally within the superior colliculus, with peripheral vision progressing caudally. It is important to note that the superior colliculus of galagos contains projections only from the contralateral visual hemifield, while the superior colliculus of gray squirrels and tree shrews receives input from all parts of the contralateral retina and, therefore, contain a small representation of the ipsilateral visual hemifield at the most rostral extent (Lane et al., 1973; Kaas et al., 1974) (Fig. 1.6). The visuotopic organization of the superior colliculus has been useful in guiding our understanding of the visuotopic organization patterns of other visual structures (Symonds and Kaas, 1978; Baldwin et al., 2011). Using the superior colliculus as a guide for determining the topographical layout of other brain

structures can be useful, especially in small areas where electrophysiological or imaging methods are difficult to conduct. In the present report we try to take full advantage of this characteristic to help us define borders between subdivision of the pulvinar complex, as well as cortical visual areas.

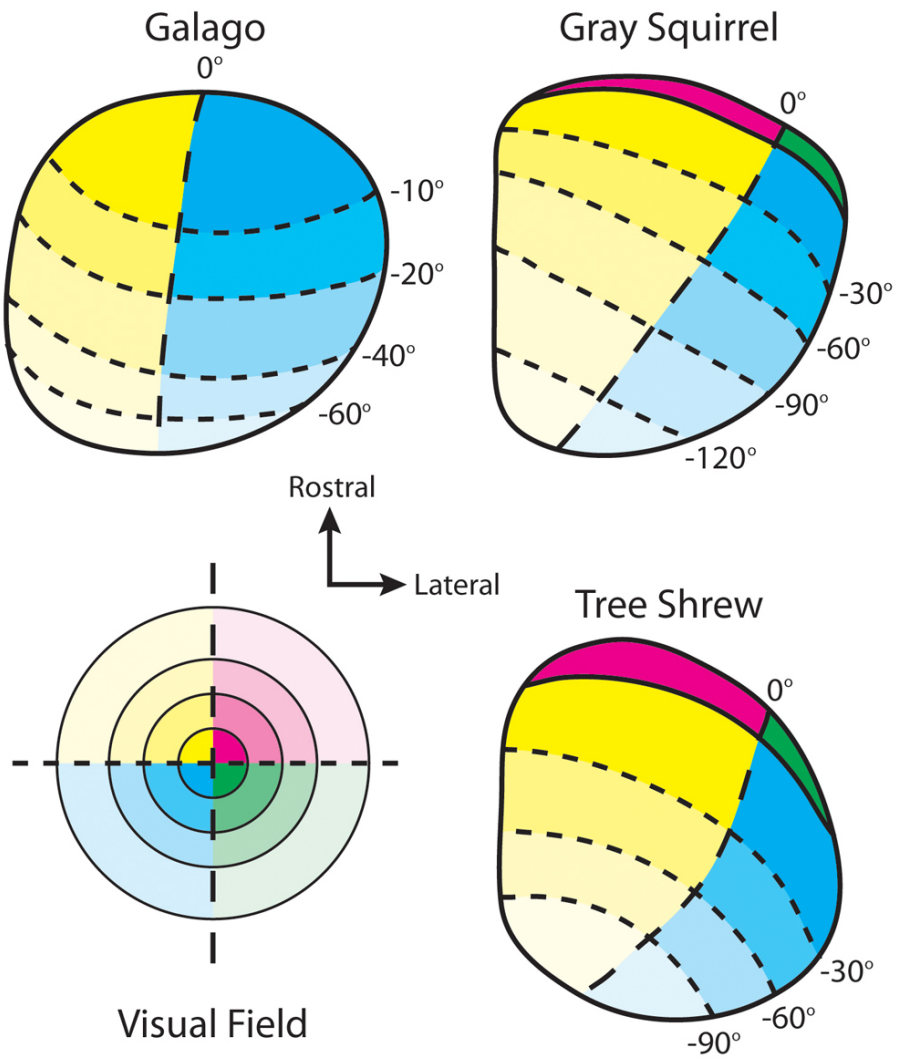

Figure 1.3. Retinotopic organization of the superior colliculus in galagos, tree shrews, and gray squirrels based on Lane et al., (1971, 1973). Central vision is represented rostarlly with peripheral vision represented caudally. The lower visual field is represented laterally, and the upper visual hemifield is represented medially.

1.3. The Extrageniculate Pathway to cortex

The extrageniculate pathway provides an alternate route of visual information to extrastriate cortex that bypasses the lateral geniculate nucleus and primary visual (striate) cortex (Fig. 1.7). This pathway travels from the retina, through the superior colliculus, to the pulvinar, and then to cortical visual areas outside of striate cortex, and appears to be present in all mammals (Harting et al., 1973). This pathway has been associated with blindsight in humans, also known as unconscious vision (Poppel et al., 1973; Stoerig and Cowey, 2007; Tamietto et al., 2010). However, in other mammals such as squirrels and tree shrews, the extrageniculate pathway may play a larger role in visual processing (Snyder and Diamond, 1968; Levey, 1973; Casagrande and Diamond, 1974; Wagor, 1978; Diamond, 1976).

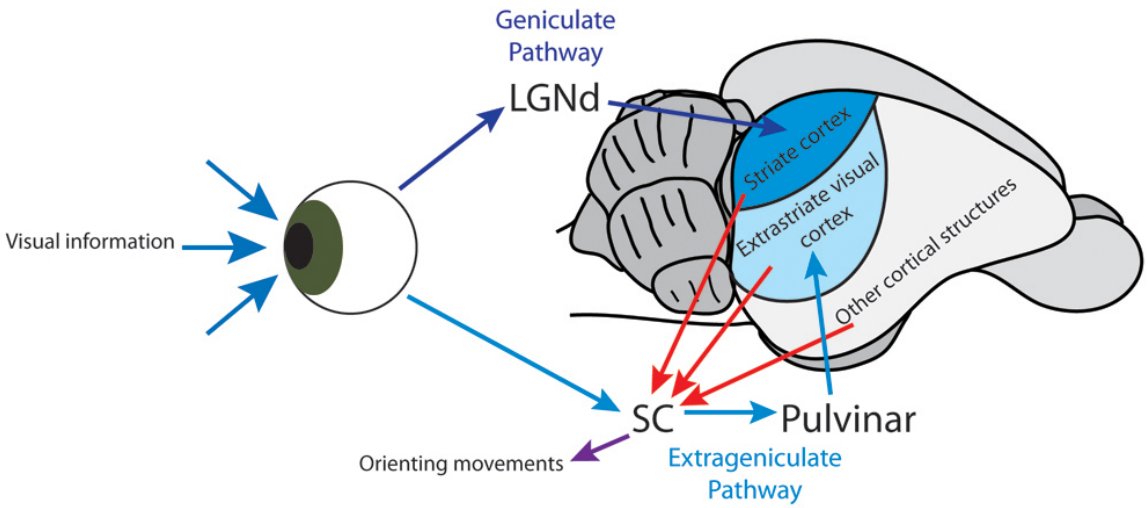

Figure. 1.4. Generalized representation of the geniculate (dark blue arrows) and extragenicuate (light blue arrows) pathways. LGNd is the dorsal lateral geniculate nucleus, SC is the superior colliculus.

The main foci of the current report were to study the projection patterns from the superior colliculus to the pulvinar complex in gray squirrels and galagos. Such studies

have been carried out in the mid to late 1970's (gray squirrels: Robson and Hall 1977; galagos: Glendenning et al., 1975). However, since that time, new staining techniques and anatomical tracers to study neuronal projections have been developed. Additionally, our understanding of the organization of the pulvinar complex in anthropoid primates has been enhanced substantially (See Kaas and Lyon, 2007; Jones, 2007 for reviews). Because galagos are prosimian primates and thought to be the least derived species within the primate lineage, they could serve as a good model for understanding the organization of the pulvinar complex in the common ancestor of primates (Kaas, 2007). Additionally, gray squirrels, being highly visual mammals with a well-developed visual system and an especially large and complex pulvinar (see Van Hooser 2006 for review), serve as a good comparative rodent model to primates. Therefore, in the current study we take advantage of the retinotopic organization of the superior colliculus (Lane et al., 1971, 1973), as well as more recent histological and immunohistological staining procedures to reveal the organization of the pulvinar complex in gray squirrels and galagos. In doing so, we are able to assess common features of the pulvinar complex between rodents and primates, as well as reveal specializations of the pulvinar complex that may reflect changes in cortical areas, such as the expansion of temporal visual areas in primates (Northcott and Kaas, 1995; Kaas, 2005, 2008, 2012).

1.4. VARIATIONS IN CORTICAL AREAS AND CORTICOTECTAL PROJECTIONS

The three species studied in the current report were not only chosen because of their similarities in ecological niches, dependence on vision, or their placement within the phylogenetic tree, but also because much is known about their cortical architecture (Wong and Kaas, 2008, 2009, 2010) (Fig. 1.8), and many characteristics of their visual cortical and other sensory cortical areas (squirrels: Hall et al., 1971; Kaas et al., 1972; Merzenich et al., 1976; Sur et al., 1978; Nelson et al., 1979; Krubitzer et al., 1986; Luethke et al., 1988—tree shrews: Sur et al., 1980, 1981; Sesma et al., 1984; Lyon et al., 1998; Remple et al., 2006, 2007; Chomsung et al., 2010—galagos: Rosa et al., 1997; Wu et al., 2003; Lyon and Kaas, 2002, Xu et al., 2005; Fang et al., 2005; Stepniewska et al., 2005, 2009a,b; Kaskan and Kaas, 2007). Though the cortex of galagos, tree shrews, and gray squirrels may have many similarities, they also have significant differences. For example, galagos have more cortical areas than tree shrews and squirrels (Wong and Kaas, 2008, 2009, 2010), and many of these areas are associated with visual processing or visuomotor behaviors which are located in the expanded temporal and parietal lobes (Kaskan and Kaas, 2007; Stepniewska et al., 2005; Fang et al., 2005). Since such cortical areas are likely involved in providing valuable information for making orienting movements, it would not be surprising that they would have corticotectal connections.

By understanding the cortical structures that project to the various layers of the superior colliculus, we can gain knowledge of how the corticotectal network is organized, and advance our understanding of the functional implications of this network. Additionally, since we will be studying both primate and non-primate Euarchontoglires species, we shall also be able to address how the expansion of cortical visual and

visuomotor areas within the temporal, posterior parietal, and frontal cortices found in primates have been incorporated into the corticotectal network.

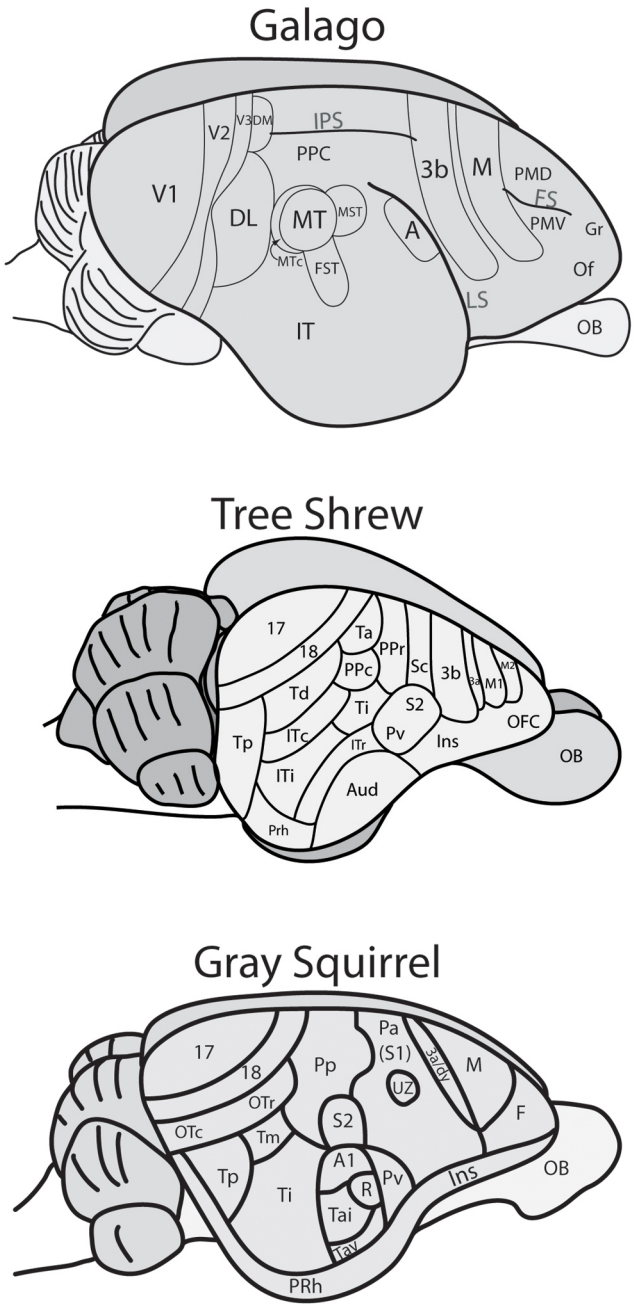

Figure 1. 5. Cortical organization schemes for galagos, tree shrews, and gray squirrels adapted form cortical maps by Wong and Kaas (2008, 2009, 2010).

1.5. SPECIFIC AIMS

The following dissertation examines the connections of the superior colliculus in three species, gray squirrels, tree shrews, and galagos. Such connections have been broken down into analyzing the projections from the superior colliculus to the pulvinar complex, which will be described in chapters two and three, and studying projections from cortex to the superior colliculus, which are described in chapters four, five and six. The specific aims of these studies address:

2. Determining the subcortical connections of the superior colliculus in gray squirrels and galagos, with the goal of gaining insights into the organization of the visual pulvinar as well as assessing possible homologous structures within the pulvinar complex between rodents and primates.
3. Understanding the corticotectal projections in gray squirrels, tree shrews, and galagos, and gaining insights into how modifications of cortex, such as the expansion of temporal visual and parietal visuomotor regions are reflected in tectal inputs.

In summary, the chapters following are directed at understanding the role of the superior colliculus in both the extrageniculate pathway, as well as its role in directing orienting movements towards visual objects of interest by way of analyzing the connectional properties of this structure. By studying such pathways in three different species within the Euarchontoglires clade, we can gain an understanding of how the extrageniculate pathway has evolved to address the expansion of cortical visual and

visuomotor areas through changes to the tectopulvinar projection patterns, as well as how and if the superior colliculus has incorporated these new cortical inputs in primates.

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CHAPTER 2

SUPERIOR COLLICULUS CONNECTIONS WITH VISUAL THALAMUS IN GRAY SQUIRRELS (*SCIURUS CAROLINENSIS*: EVIDENCE FOR FOUR SUBDIVISONS WITHIN THE PULVINAR COMPLEX[1]

2.1. ABSTRACT

As diurnal rodents with a well-developed visual system, squirrels provide a useful comparison of visual system organization with other highly visual mammals such as tree shrews and primates. Here, we describe the projection pattern of gray squirrel superior colliculus (SC) with the large and well-differentiated pulvinar complex. Our anatomical results support the conclusion that the pulvinar complex of squirrels consists of four distinct nuclei. The caudal (C) nucleus, distinct in cytochrome oxidase (CO), acetylcholinesterase (AChE), and vesicular glutamate transporter 2 (VGluT2) preparations, received widespread projections from the ipsilateral SC, although a crude retinotopic organization was suggested. The caudal nucleus also received weaker projections from the contralateral SC. The caudal nucleus also projects back to the ipsilateral SC. Lateral (RLl) and medial (RLm) parts of the previously defined rostral lateral pulvinar (RL), were architectonically distinct, and each nucleus received its own retinotopic pattern of focused ipsilateral SC projections. The SC did not project to the

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rostral medial (RM) nucleus of the pulvinar. SC injections also revealed ipsilateral connections with the dorsal and ventral lateral geniculate nuclei, nuclei of the pretectum, and the nucleus of the brachium of the inferior colliculus, and bilateral connections with the parabigeminal nuclei. Comparisons with other rodents suggest that a variously named caudal nucleus, that relays visual inputs from the SC to temporal visual cortex, is common to all rodents, and possibly most mammals. RM and RL divisions of the pulvinar complex, also appear to have homologues in other rodents.

2.2. INTRODUCTION

The pulvinar is a part of an extrageniculate visual pathway where information is passed from the retina to the superior colliculus (SC), and then to the pulvinar complex that in turn projects to extrastriate visual cortex. This alternate pathway of transmitting visual information to cortex appears to be present in all mammals (Harting et al., 1973a), and it provides a means for extrastriate cortex to receive retinal input other than from the relay through the lateral geniculate nucleus (LGN) and primary visual cortex (V1). In humans, this extrageniculate pathway to cortex via the superior colliculus and pulvinar is thought to play a critical role in the unconscious processing of visual information (blind sight; Poppel et al., 1973; Stoerig and Cowey, 2007; Tamietto et al., 2010). In squirrels, the geniculate visual pathway sends information largely or completely to V1 (Kaas et al., 1972b), but many visual abilities are preserved after large lesions of V1 when extrastriate cortex remains intact (Levey, 1973; Wagor, 1978). Thus, the extrageniculate pathway to cortex via the SC plays a large role visual perception in squirrels (Diamond, 1976).

Several characteristics of the SC in squirrels are consistent with an enhanced role in vision. The SC of squirrels is well laminated and is among the largest in all studied mammals (LeGros Clark, 1959; Lane et al., 1971; Kaas and Collins, 2001). The pulvinar is also quite large in grey squirrels (Abplanalp, 1970; Robson and Hall, 1977). Initially the pulvinar of squirrels was thought to be a homogeneous structure (Abplanalp, 1970). However, subsequent studies of connections and architecture revealed that the squirrel pulvinar can be divided into a least 3 subdivisions; the caudal subdivision (C), with diffuse inputs from SC and large Nissl stained cell bodies, a rostral lateral subdivision (RL), with more specific projections from SC and “patchy” clusters of Nissl stained

bodies, and a rostral medial (RM) subdivision, with no apparent connections with the SC (Robson and Hall, 1977).

In the present study, we determined retinotopic projection patterns of the SC to architectonic divisions of the pulvinar in squirrels. We obtained further evidence for the three previously proposed divisions of the pulvinar and, unexpectedly, found evidence for an additional fourth division. Projections from the SC to visual thalamus were studied by injecting anatomical tracers into the superior colliculi of five grey squirrels. Results were related to subdivisions of the visual thalamus that were revealed in sections processed for Nissl substance, cytochrome oxidase (CO), acetylcholinesterase (AChE), or the vesicular glutamate transporter 2 (VGluT2). Squirrels were of special interest in this study because their well developed visual system (Kaas, 2002; Van Hooser, 2006) provides a useful model that affords an understanding of visual system organization that may apply to other rodents, such as rats and mice, as well as revealing convergent specializations with the visual systems of other highly visual mammals (Kaas, 2002). Additionally, as rodents and primates are within the Euarchontoglires clade of eutherian mammals (Murphy et al., 2001), they are likely to share many features of visual system organization.

2.3. MATERIALS AND METHODS

2.3.1. Animals

Nine grey squirrels weighing between 400 and 560g were used for the current study. Five squirrels received tracer injections, three were processed for additional architectonic information, and one additional squirrel was used for western blot analysis of VGluT2. All surgical procedures were carried out in accordance with the NIH *Guide for the Care and Use of Laboratory Animals* under a protocol approved by the Vanderbilt University Animal Care and Use Committee.

2.3.2. Surgery and Injections

All surgeries were conducted under aseptic conditions in anesthetized animals. Grey squirrels were initially anesthetized with an intramuscular (IM) injection of ketamine hydrochloride (120 mg/kg) and of xylazine (8 mg/kg). Lidocaine was placed in both ears and the head was secured within a stereotaxic frame. Anesthesia was maintained during surgery using 0.5-2% isoflurane delivered through a facemask. An incision was made along the midline of the skull, and a small craniotomy was made to expose the left parietal and occipital cortex. The dura was then cut and reflected. In four cases, portions of the left occipital pole and parietal cortex were aspirated in order to reveal the left superior colliculus. In three of these cases (09-02, 09-23, 09-44), tracer injections were placed in the left SC, while in the other case (09-50), injections of anatomical tracers were placed into the right SC after partial retraction of the intact right hemisphere. Finally, in one case, injections of anatomical tracers were placed after the SC was visualized, not by aspiration, but by retraction of the occipital pole and cerebellum (case 04-15). Cholera toxin B-subunit (CTB, Molecular Probes Invitrogen, Carlsbad, CA,

10% in distilled water), and Fluoro Ruby (FR, Molecular Probes Invitrogen, Carlsbad, CA, 10% in distilled water) tracers were pressure injected into the SC by a Hamilton syringe fitted with a glass pipette beveled to a fine tip. Volumes of 0.40 to 0.60ml of tracer were injected at various retinotopic locations within the SC at depths ranging from 0.7mm to 1.1mm from the surface of the SC. The needle tip was left at each site for 5 minutes to allow for tracer diffusion. Any leakage of the tracer to the SC surface during injection was removed with sterile saline flushes in order to prevent tracer contamination of surrounding brain tissue.

After injections of anatomical tracers were placed into the SC, gelfoam was placed in the region of aspirated cortex, and gelatin film was placed between the brain and skull. The opening of the skull was sealed with an artificial bone flap made of dental cement and the incision site was closed with surgical staples. Animals were then carefully monitored during recovery from anesthesia. Once squirrels were fully awake, they were given Buprenex (0.3mg/kg IM) analgesic and were returned to their home cage with food and water.

2.3.3. Histology

Three to six days after surgery, animals were injected with a lethal dose of sodium pentobarbital (80mg/kg), and were perfused with phosphate-buffered saline (PBS; pH 7.4) followed by 2% paraformaldyhyde in PB and 2% paraformaldyhyde in PB with 10% sucrose. The brain was removed, and cortex was separated from thalamus and brainstem. The right cerebral cortex was artificially flattened and processed as part of another study, while the thalamus and brainstem were submerged in 30% sucrose solution for cryoprotection overnight or up to 48 hours.

The thalamus and brainstem were cut in the coronal plane at 40um thickness on a freezing microtome and saved in several series depending on the number of tracers injected and the planned immunohistochemical staining procedures. One architectonic case was cut along the horizontal plane. In cases with FR injections, a series of one in five sections was mounted directly onto glass slides without further processing. For cases with CTB injections, a one in five series of sections was processed using an immunohistochemical protocol to reveal injection sites, labeled cell bodies, and axon terminals. Sections were rinsed in phosphate-buffered saline (PBS, pH 7.2) and then were incubated with 5% normal rabbit serum, and 0.5% Triton X-100 in PBS for 2 hours at room temperature. Sections were then incubated in PBS containing a goat anti-CTB antibody (List Biological Laboratories: lot No. 7032A3: 1:5000), 0.5% Triton X-100, and 5% normal rabbit serum for 48 hours at 6^{o}C. Sections were then rinsed thoroughly in PBS, and then incubated in anti-goat biotinylated antibody (PK-4005 kit: Vector Laboratories, Burlingame, CA, 1:200), 0.5% Triton X-100, and 5% normal rabbit serum in PBS for 90 minutes at room temperature. After sections were rinsed thoroughly in PBS, they were incubated in an avidin-biotin-peroxidase complex (PK-4005 kit; Vector Laboratories, Burlingame CA, 1:80) with 0.05% Triton X-100 in PBS for two hours at room temperature. Sections were then rinsed thoroughly in PBS followed by rinses in tris buffer (TB, pH 7.5). CTB-peroxidase was visualized by a reacting the tissue in a 3.3'-diaminobenzidine tetrahydrochloride (DAB; 50mg/100ml) containing H_2O_2 (0.15ul/50ml) and 0.03% nickel ammonium sulfate in TB. Sections were then mounted, dehydrated, and coverslipped.

To reveal architectonic features of thalamic nuclei and subnuclei, additional series were processed for traditional histochemical markers for Nissl substance (thionin), cytochrome oxidase (CO; Wong-Riley, 1979), and acetylcholinesterase (AChE; Geneser-Jensen and Blackstad, 1971), as well as for the immunohistochemical marker for vesicular glutamate transporter 2 (VGluT2; mouse monoclonal anti-VGluT2 from Chemicon, now part of Millipore, Billerica, MA; 1:5000). VGluT2 is a general maker of subcortical projections to the dorsal thalamus, as well as thalamocortical projections to sensory cortex (Herzog et al., 2001; Hackett and de la Mothe, 2009; Wong and Kaas, 2009) and has been used to differentiate nuclei within the pulvinar (Chomsung et al., 2008).

2.3.4. Western Immunoblots

Western blot analysis was performed for the VGluT2 antibody using fresh frozen brain tissue from one grey squirrel. The grey squirrel was initially anesthetized with an intramuscular (IM) injection of ketamine hydrochloride (120 mg/kg) and then was given a lethal dose of sodium pentobarbital (80mg/kg). The brain was quickly removed, sectioned and frozen at -80^{o}C for three days. A brain section containing cerebellar tissue was placed in ice-cold lysis buffer (pH 7.2) containing 0.32 M sucrose, 2mM EDTA, 1% SDS, 50uM PMSF, 1 ug/mL leupeptin, and Roche Complete® protease inhibitor. A Kontes pellet mortar and pestle was used to homogenize the tissue after which, samples were centrifuged at 17 000 g for 10 minutes. Protein concentrations of the supernatant were determined using the BCA method. Forty micrograms of protein was run on 8% acrylamide gels and transferred to PVDF membranes. Membranes were washed with tris buffered saline (TBS, pH 8.0) with 0.01% Triton X-100, then transferred to a 5% BSA

blocking solution in TBS-Triton X-100 for an hour. Membranes were then transferred to 1:1000 dilution of VGluT2 primary antibody in TBS with 0.1% Triton X-100 and 5% BSA for 24 hours at 4 ^{o}C. Membranes were rinsed with TBS and 0.1% Triton X-100 several times, and then incubated in goat anti-mouse (1:20 000 dilution for VGluT2, Jackson Immuno Laboratories, USA) for an hour at room temperature followed by several washes in TBS with 0.1% Triton X-100. Protein was visualized using chemiluminescence and exposure of membranes to film. The film was then scanned and presented in Fig. 2.1.

2.3.5. Antibody Characterization

Table 2.1 lists all antibodies used. The CTB antibody was tested on squirrel brain tissue with no CTB injections. This control failed to label any cells or patches of axon terminals.

Western blot analysis was performed using the VGluT2 antibody. Our analysis showed a single band of labeled protein at around 56 kDa, the molecular weight of VGluT2 (Fig. 2.1). Additionally the staining pattern from VGluT2 within squirrel thalamus, specifically within the medial geniculate nucleus (MGN), was comparable to those previously reported (Wong et al., 2008).

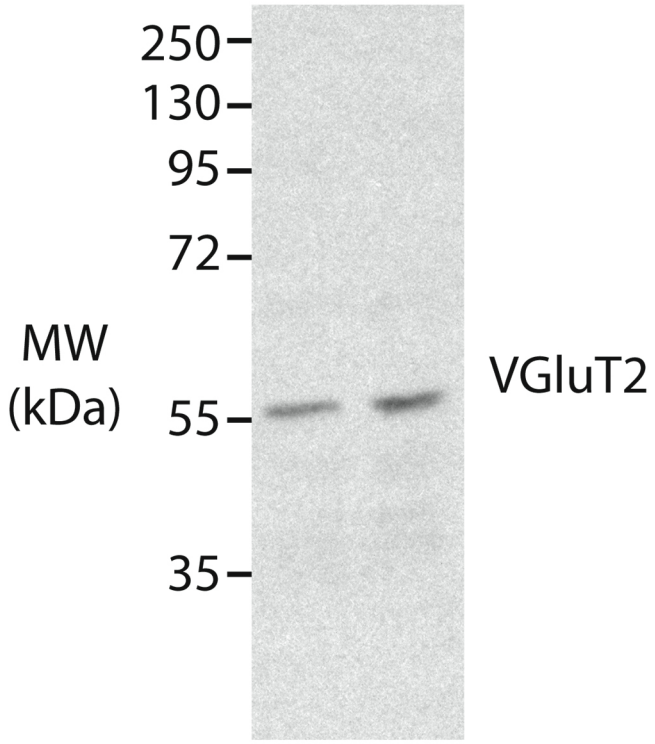


Figure 2.1. ***Western Blot characterization of VGLUT2 antibody****. The VGLUT2 antibody recognizes a 56-kDa protein in gray squirrel cerebellar lysate, which is the expected molecular weight of the VGLUT2 protein.*

2.3.6. Data Analysis

The locations of anterogradely labeled axon terminals, and retrogradely labeled cell bodies, were plotted using a Neurolucida system (MicroBright Field, Williston, VT). Digital images of processed sections were taken using a DXM1200F digital camera mounted to a Nikon E800S microscope (Nikon Inc., Melville, NY). Photomicrographs were adjusted for brightness and contrast using Adobe Photoshop (Adobe Systems Inc.), but were otherwise unaltered.

Series of sections stained for various architectonic markers were used to locate injection sites, anterograde and retrograde tracer label, and thalamic borders. Injection site locations relative to SC layers were determined using CO, Nissl, and VGluT2 stained

sections, while subdivisions of thalamic nuclei were delineated within Nissl, CO, AChE, and VGluT2 stained sections. Drawings of nuclear boundaries in thalamic sections were aligned with tracer plots of anterograde and retrograde label using common blood vessels and landmarks.

Table 2.1 Antibody Characterization

Antigen	Immunogen	Manufacturer	Dilution factor
Cholera toxin subunit B	Purified CTB isolated from Vibrio cholerae	List Biological Laboratories (Campbell, CA), goat polyclonal, No. 703	1:5,000
Vesicular glutamate transporter 2	Recombinant protein from rat VGLUT2, full length	Chemicon now part of Millipore (Billerica, MA), mouse monoclonal, No. MAB5504	1:5,000

2.4. RESULTS

The present study describes the patterns of connections between the SC and the visual thalamus of grey squirrels with the main focus on connections between the SC and the pulvinar complex. All injections involved the superficial layers, and to some extent, the intermediate layers of the SC. Connections between the SC and pulvinar revealed that the SC projects to three of four distinct subdivisions within the pulvinar complex. One projection is diffuse (or widespread) within the caudal division, while two other projections are focused and terminate in two separate locations within the previously described rostral lateral pulvinar (Robson and Hall, 1977). We provide evidence that two of the three SC projections to pulvinar are topographic and that the caudal division has reciprocal connections with SC. We first present our findings of architectonic differences between nuclei within the pulvinar complex followed by the results of our connection studies.

2.4.1. *Architectonic characteristics of the superior colliculus and thalamic nuclei*

We used a series of histochemical and immunohistochemical stains in coronal and horizontal brain sections to reveal and characterize the layers of the SC, as well as identify visual thalamic nuclei and subnuclei. These stains include those for acetylcholinesterase (AChE), Nissl substance, cytochrome oxidase (CO), and vesicular glutamate transporter 2 (VGluT2).

2.4.1.1. Superior Colliculus

The squirrel superior colliculus is a well-defined structure with at least 7 distinguishable layers (Fig. 2.2), as described previously (see Kaas and Huerta, 1988; May, 2006). However, our results allowed three sublayers to be distinguished within the stratum griseum intermedium (SGI), which is comprised of ventral and dorsal CO dense sublayers separated by a CO weak sublayer. A similar pattern was visualized using VGluT2 staining, with strong VGluT2 staining dorsal and ventral to a weak VGluT2 staining sublayer (Fig. 2.2). AChE and Nissl preparations did not distinguish the three sublayers. However, dark AChE staining did coincide with the most dorsal CO dark band within the SGI, and Nissl stained cells appeared to be larger within the ventral darkly stained CO and VGluT2 layers but not within the dorsal or weakly stained CO and VGluT2 layers (Fig. 2.2). These three sublayers are apparent over most of the rostrocaudal extent of the SC. A similar lamination pattern has been observed in unstained wet rat SC tissue preparations (Helms et al., 2003) and thus we use a similar nomenclature in the present study.

2.4.1.1. The Pulvinar Complex

2.4.1.1.1. Caudal pulvinar

The caudal pulvinar lies caudal and medial to the LGNd, and is distinguished from the surrounding thalamic nuclei by a dense population of Nissl-stained neurons with large cell bodies (also see Robson and Hall, 1977) and a dark appearance in AChE, CO, and VGluT2 preparations (Fig. 2.3 and Fig. 2.4). In addition, the caudal pulvinar is lightly myelinated (not shown). At the most posterior end of the pulvinar complex, only

the caudal pulvinar is present (Fig. 2.3A, E, I, M, and Fig. 2.4); however, in more anterior positions the caudal pulvinar is found more dorsally as RL begins to emerge (Fig. 2.3B, C, F, G, J, K, N, O).

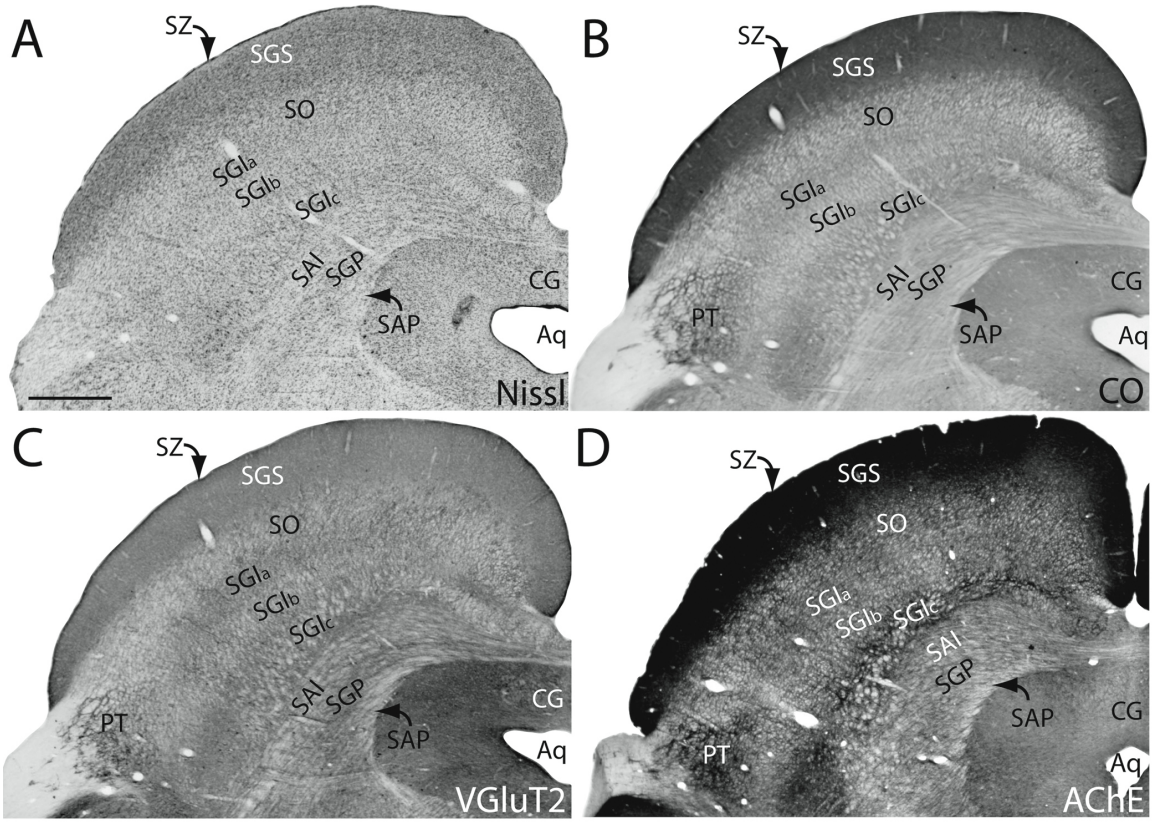
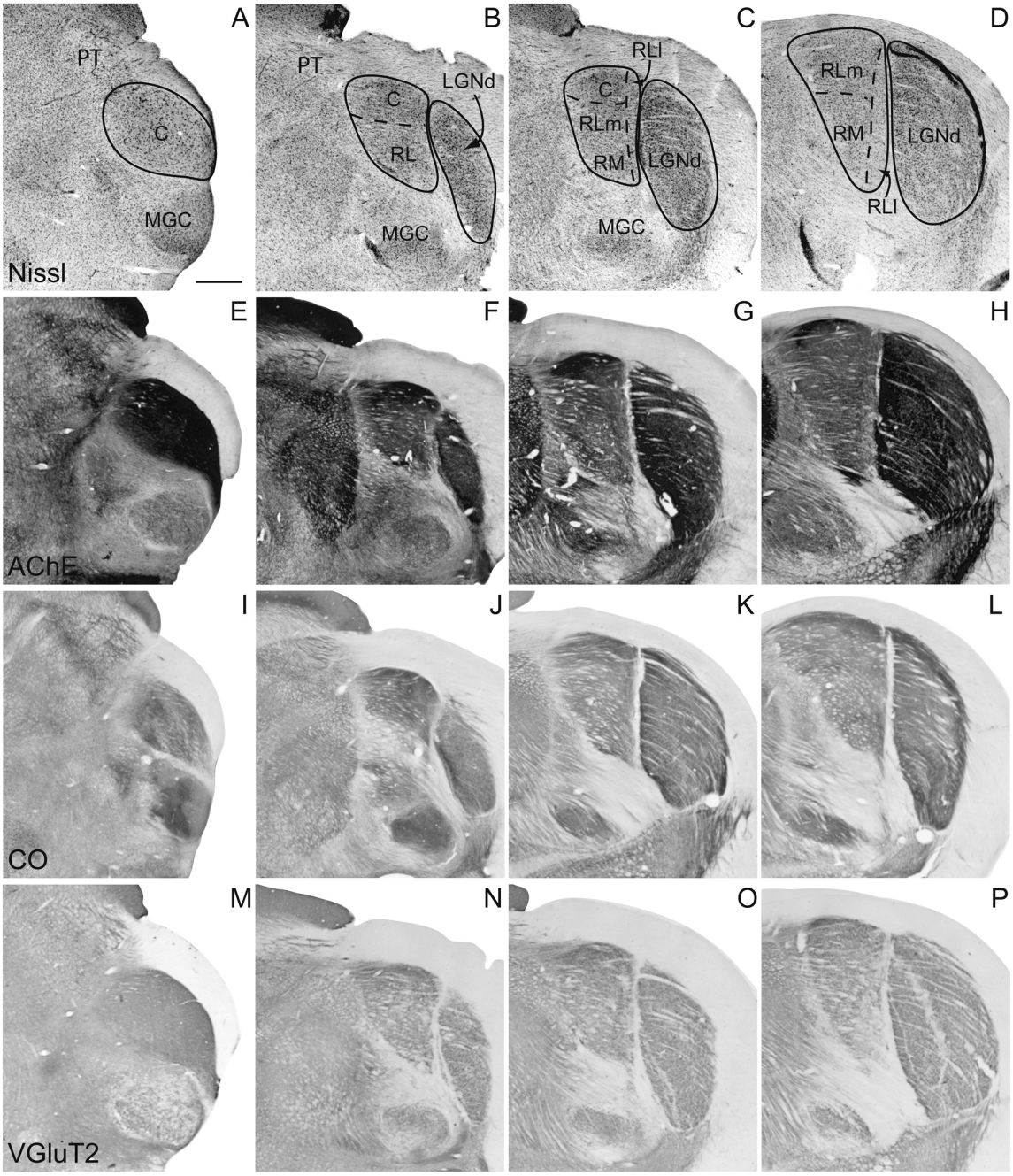


Figure 2.2 ***Architecture of the superior colliculus in gray squirrels****. Photomicrographs of coronal sections through the superior colliculus of the gray squirrel after staining with Nissl (**A**), cytochrome oxidize (CO; **B**), vesicular glutamate transporter-2 (VGluT2; **C**), and acetylcholinesterase (AChE; **D**). Seven layers can be distinguished from one another by using each of these stains. SZ, stratum zonale; SGS, stratum griseum superficiale; SO, stratum opticum; SGI, stratum griseum intermedium; SAI, stratum album intermedium; SGP, stratum griseum profundum; SAP, stratum album profundum. Note the presence of three possible layers within the SGI. Images were taken from two squirrels: Nissl, CO, VGluT2 are from the same squirrel; the AChE photomicrograph is from a second squirrel. Scale bar . 1 mm.*

Figure 2.3 ***Architectonic characteristics of subdivisions within the gray squirrel pulvinar complex****. Coronal sections through various stages of the pulvinar complex were stained for Nissl substance (A–D), acetylcholinesterase (AChE; E–H), cytochrome oxidase (CO; I–L), and vesicular glutamate transporter-2 (VGluT2; M–P). Sections on the left are more caudal and progress to more rostral sections on the right. The borders of proposed subdivisions within the pulvinar complex are shown with dashed lines. Images were taken from two squirrels: Nissl, AChE, and VGluT2 are taken from the same squirrel, whereas the CO images are taken from a second squirrel. Scale bar . 1 mm.*


A
PT
C
MGC
Nissl
B
PT
LGNd
C
RL
MGC
C
RLl
C
RLm
RM
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D
RLm
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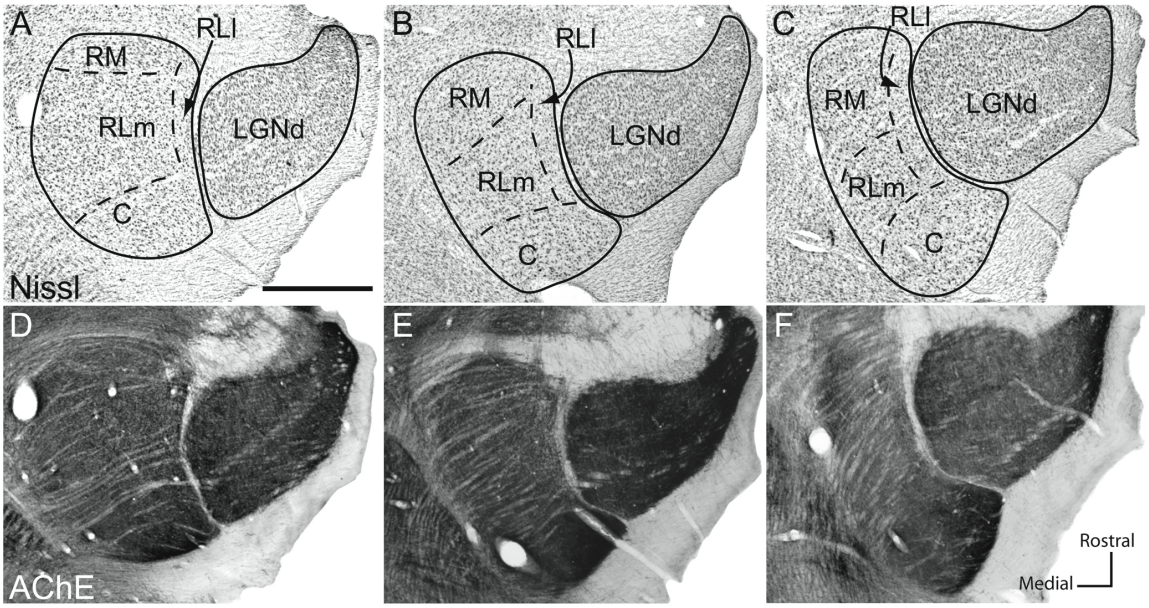


Figure 2.4. ***Architectonic characteristics of subdivisions within the gray squirrel pulvinar complex****. Horizontal sections through various stages of the pulvinar complex were stained for Nissl substance (A–C) and acetylcholinesterase (AChE; D–F). Sections on the left are more dorsal and progress to more ventral sections on the right. The borders of proposed subdivisions within the pulvinar complex are shown with dashed lines. Scale bar . 1 mm.*

2.4.1.1.2. Rostral lateral pulvinar

The rostral lateral pulvinar (RL) is dorsal medial to the LGNd, ventral to the caudal pulvinar, and dorsal lateral to RM. We have defined two subdivisions within RL. The lateral subdivision, RLl, is long and thin, and lies on the most lateral border of the pulvinar next to the LGNd (Fig. 2.3, and Fig. 2.4). In AChE stains, long vertical fibers course through RLl (Fig. 2.5). This subdivision has a lower density of Nissl stained cell bodies with respect to the surrounding pulvinar (Fig. 2.3C, D, and Fig. 2.4).

The medial subdivision within the rostral lateral pulvinar (RLm) is moderately populated with Nissl-stained cell bodies that are smaller in size relative to those in the caudal subdivision, as noted by Robson and Hall (1977), and those in the RM subdivision. AChE fibers course mediolaterally (Fig. 2.5), and the AChE staining, in

general, is weaker than that of the caudal division. CO, and VGluT2 preparations do not clearly differentiate between RLm and RLl, although RLl stains lighter than RLm in VGluT2 preparations (Fig. 2.3O, P).

2.4.1.1.3. Rostral medial pulvinar

The rostral medial pulvinar is located ventral and medial to the rostral lateral pulvinar. This division of the pulvinar complex has patches or clusters of Nissl-stained cell bodies (Fig. 2.3C, D, and Fig. 2.5C; also see Robson and Hall, 1977). In AChE preparations, the fibers are slightly finer than those in RL and course in a medial to lateral direction (Fig. 2.3G, H), which helps in distinguishing RM from RLl. The density of AChE, CO, and VGLUT2 staining is similar between RM and RLm (Fig 2.3-5) making it difficult to determine the architectonic border between RM and RLm.

2.4.1.1. Other Subcortical Visual Nuclei

2.4.1.1.1. Dorsal lateral geniculate nucleus

The dorsal lateral geniculate nucleus (LGNd) in grey squirrels is located ventrolateral to the pulvinar complex. In Nissl preparations, the LGNd is differentiated from surrounding structures by its densely packed and darkly stained cell bodies. Three architectonically defined layers are present in squirrel LGNd but as many as six layers can be determined when studying ipsilateral and contralateral retinal inputs. These layers are 0, 1, 2, 3a, 3b, and 3c (Tigges, 1970; Kaas et al., 1972b; Cusick and Kaas, 1982), with layer 0 being provisionally recognized (Major et al., 2003; Cusick and Kaas, 1982). In the current study, layers 1, 2, and 3 were separated from one another by cell sparse zones.

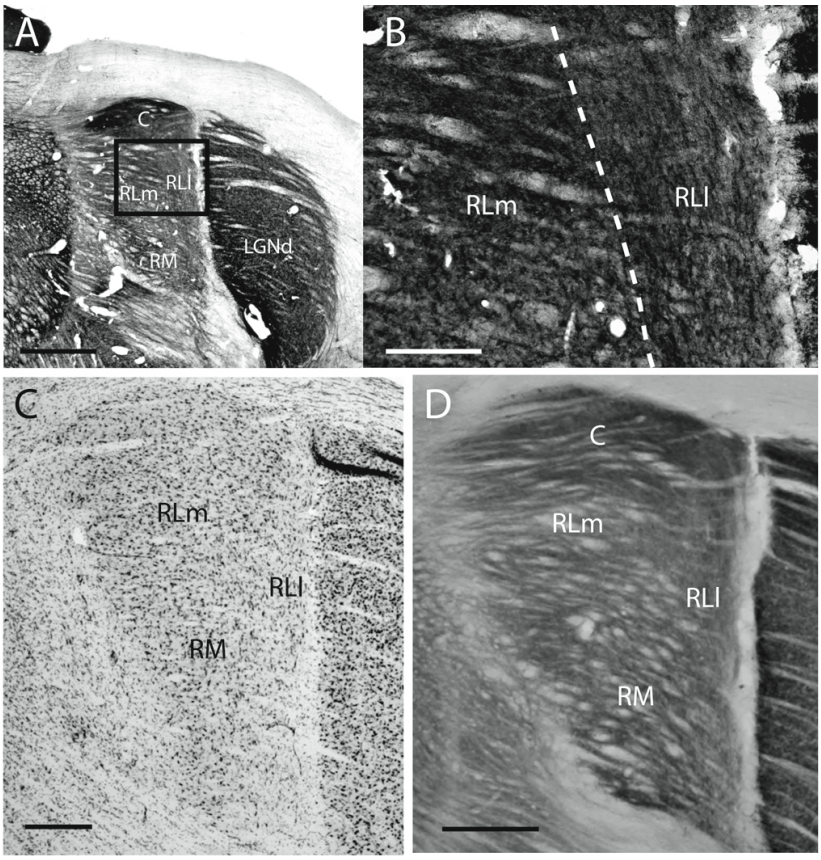


Figure 2.5. Photomicrographs of a coronal section of the pulvinar complex in gray squirrel stained for AChE. B is a higher magnification image of the boxed area in A. Note the difference in the direction of fibers between rostral lateral medial (RLm) and rostral lateral lateral (RLl). C is an image of a Nissl-stained section; D is an image of a cytochrome oxidase-stained section. Scale bars . 1 mm in A; 250 lm in B; 0.5 mm in C,D.

These cell sparse zones are easily identified in VGluT2 preparations (Fig. 2.3O, P). Layer 1makes up a majority of the medial border (Fig. 2.3D, P) of the LGNd and layer 3 is located along the lateral border of the LGNd, adjacent to the optic tract.

2.4.1.1.2. Ventral lateral geniculate nucleus

The LGNv can be differentiated from surrounding thalamus based on strong CO, AChE, and VGluT2 staining (Fig. 2.3). The ventral zone of the LGNv has commonly been divided into two main layers: an internal or medial layer, also known as the

nonretinal recipient layer, that has small pale Nissl stained cells; and an external or lateral layer, also known as the retinal recipient layer, of larger darker Nissl staining cells (May, 2006). The lateral layer can be distinguished from the medial layer by its relatively dark AChE, CO, and VGluT2 staining (Fig. 2.6). The LGNv also includes a dorsal cap, and the intergeniculate leaflet (Major et al., 2003; Smale et al., 1991). The IGL stains darkly for AChE, and is separated from the LGNv by a septum that does not express VGluT2 (Fig 2.6). In our drawings of label in the LGNv, we did not identify lateral, medial and IGL divisions.

2.4.1.1.1. Pretectum

The pretectum (PT) in squirrels has been subdivided into 4 nuclei (Major et al., 2003). In this paper, PT corresponds to the nucleus of the optic tract (NOT), and possibly parts of the posterior pretectal nucleus (PPN) described by Major et al., 2003. The PT complex is medial to the pulvinar complex. Caudally, the PT is wedged between the intermediate and deep layers of the superior colliculus, and then extends into the brachium of the SC more rostrally. This group of PT nuclei can be differentiated from the SC and pulvinar by strong AChE and CO staining relative to surrounding tissue (Fig. 2.2). Additionally, the fibers in this area are less dense relative to the surrounding SC tissue and are arranged in a reticular pattern.

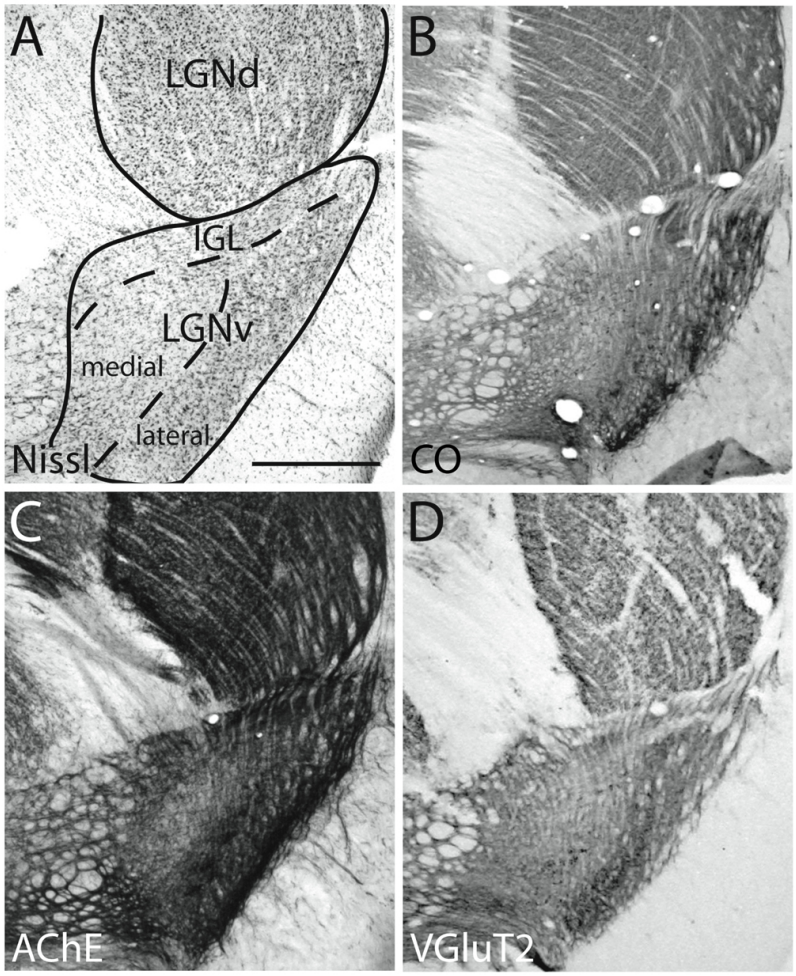


*Figure 2.6. Photomicrographs of coronal sections of the ventral lateral geniculate nucleus (LGNv) in gray squirrels stained for Nissl substance (**A**), cytochrome oxidase (CO; **B**), acetylcholinesterase (AChE; **C**), and vesicular glutamate transporter-2 (VGluT2; **D**). The dashed line indicates the border between the medial and lateral subdivisions of the LGNv as well as the border between the intergeniculate leaflet (IGL) and the LGNv. Images were taken from two squirrels. Scale bar . 1 mm.*

2.4.1.1.1. Nucleus of the brachium of the inferior colliculus

The nucleus of the brachium of the inferior colliculus (NBIC) is dorsal to the parabigeminal nucleus and caudal to the medial geniculate nucleus. The NBIC stains moderately for AChE, VGluT2, and CO, and has large Nissl stained cell bodies (Fig 2.7).

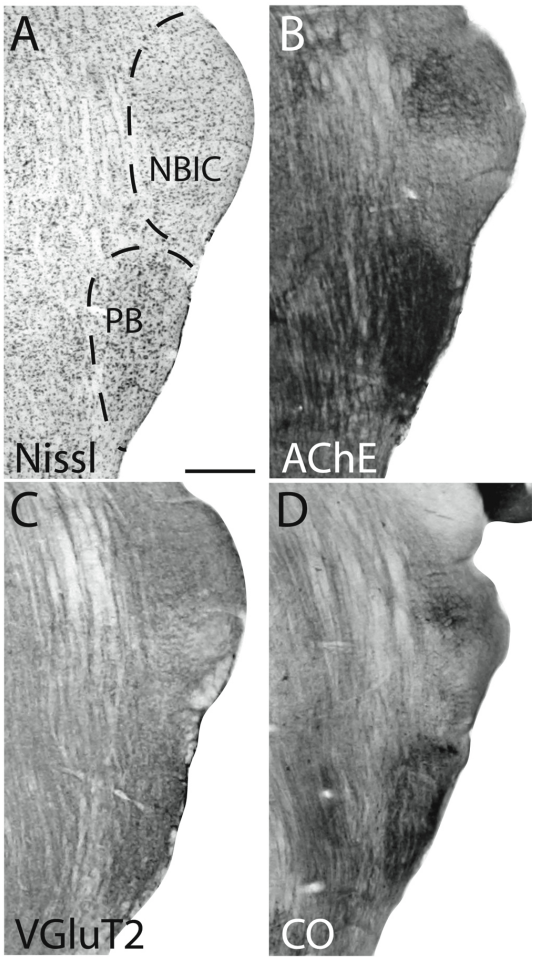


*Figure 2.7. Photomicrographs of coronal sections of the parabigeminal (PB) and the nucleus of the brachium of the inferior colliculus (NBIC) in gray squirrels stained for Nissl substance (**A**), acetylcholinesterase (AChE; **B**), vesicular glutamate transporter-2 (VGluT2; **C**), and cytochrome oxidase (CO; **D**). A–C are taken from one squirrel; D was taken from a second squirrel. Scale bar 1 mm.*

2.4.1.1.2. Parabigeminal nucleus

The parabigeminal nucleus (PB) is located along the lateral margin of the midbrain just ventral to the brachium of the inferior colliculus. In the grey squirrel the PB nucleus can be identified by a high concentration of large darkly stained Nissl cells relative to surrounding tissue. The PB also stains darkly for CO, AChE, and VGluT2 relative to surrounding tissue (Fig. 2.7).

2.4.2. Superior colliculus connections with visual thalamus

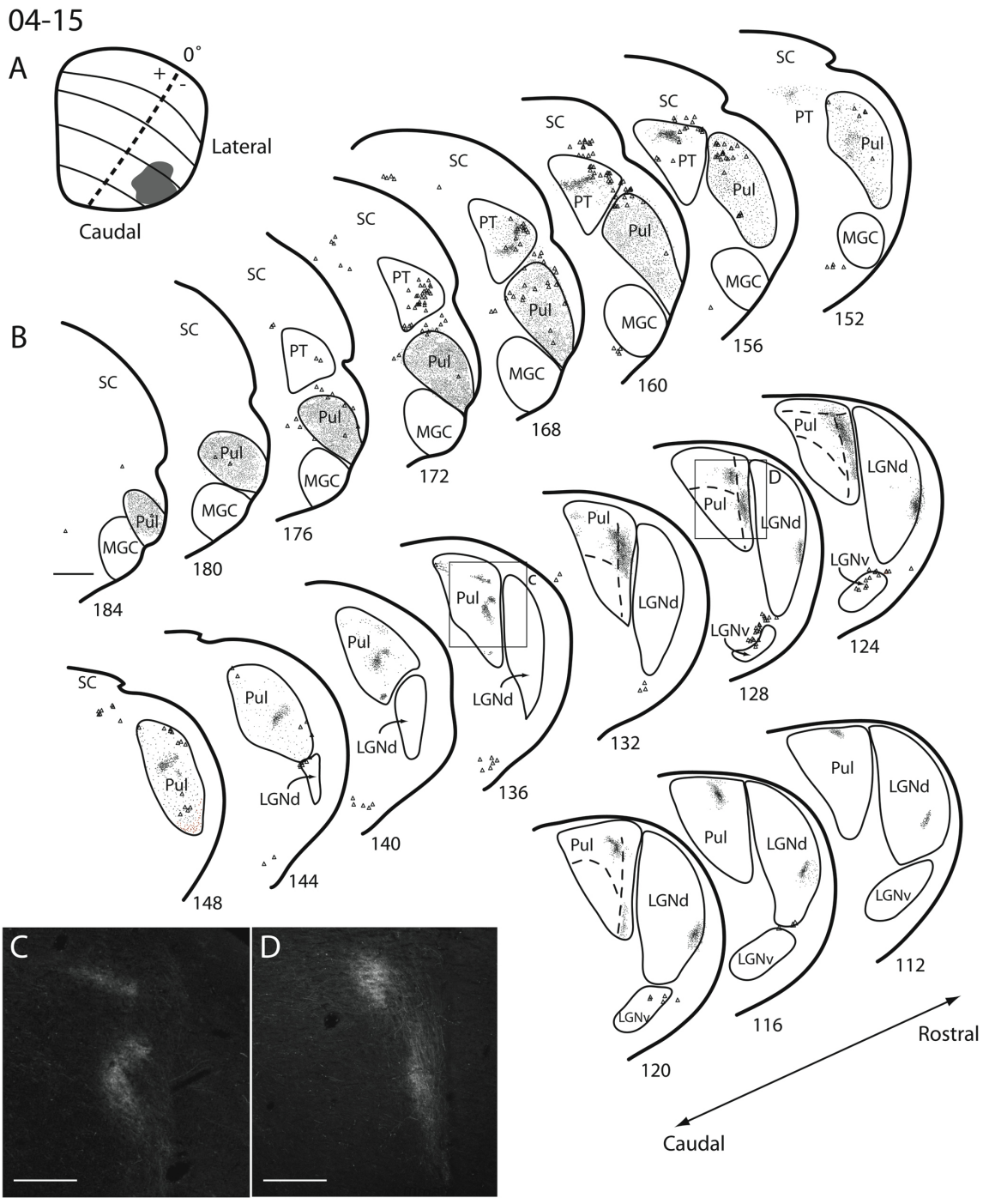
Connections of the superior colliculus with visual thalamus were studied using anatomical tracer injections and plotting both anterogradely labeled axon terminal and retrogradely labeled cell body locations.

2.4.2.1. SC Connections with Pulvinar

2.4.2.1.1. Caudal pulvinar

Injections within SC resulted in widespread terminal label within the ispsilateral caudal division of the pulvinar complex. Injections made in several locations of the superior colliculus labeled terminals throughout a large extent of the caudal pulvinar (Fig. 2.8-12). Thus, these terminals do not appear to be strongly retinotopically organized. However, there may be a crude retinotopic pattern with more caudal SC injections labeling axon terminals more caudally within the caudal pulvinar (Fig. 2.8, 2.9) and more rostral injections labeling terminals more rostrally (Fig. 2.10, 2.11, 2.12). Anterograde label after SC injections was found throughout the entire rostrocaudal extent of the caudal pulvinar as defined by Nissl, CO, AChE, and VGluT2 staining. The most rostral injections into the SC labeled terminals in the rostral aspect of the caudal pulvinar up to the most rostral border (Fig. 2.12) while the most caudal SC injection labeled terminals that extended to the caudal border of the caudal pulvinar (Fig. 2.8). In some cases, a few retrogradely labeled cells were present within the caudal division of pulvinar (Fig. 2.8, 2.9). A few labeled axon terminals were observed within the contralateral caudal pulvinar, as described by Robson and Hall (1977). No labeled cells were observed within the contralateral caudal pulvinar.

Figure 2.8. Superior colliculus (SC) connections with visual thalamus in squirrel 04-15. ***A****: The extent and estimated retinotopic position of the fluoro-ruby (FR) injection site on a reconstructed dorsal view of the SC.* ***B****: Coronal thalamus sections are arranged in caudal to rostral progression, with the most caudal section in the upper left and the most rostral section located in the lower right. Locations of labeled axon terminals are shown with dots, whereas the locations of retrogradely labeled cell bodies are represented with triangles.* ***C****: Highpower photomicrograph of terminal label within the pulvinar complex. The magnified photograph corresponds to the box located in section 136 of B.* ***D****: High-power photomicrograph of terminal label within the pulvinar complex corresponding to the box located in section 128 of B. Scale bars . 1 mm in B; 0.5 mm in C,D.*



2.4.2.1.2. Rostral lateral pulvinar

There appear to be two topographically organized projection zones within the RL pulvinar. Thus, we distinguish a lateral RL (RLl) nucleus, from a medial RL (RLm) nucleus. Projections to the RLl appear to be retinotopically organized, as injections made

more rostrally within the SC result in labeled terminals dorsal within RLl, while injections made more caudally within the SC result in labeled terminals more ventral within RLl (Fig. 2.11, and compare Fig. 2.8 to Fig. 2.12). Additionally, injections made into more medial portions of SC (representing the upper visual field) labeled terminals more laterally within RLl, while injections to more lateral locations within the SC (representing the lower visual field) labeled terminals more medially within RLl (Fig. 2.11, and compare Fig. 2.12 to Fig. 2.10). This pattern of organization suggests that frontal vision is represented more dorsally and peripheral temporal vision more ventrally within RLl. Furthermore, the lower visual field is represented nearer to the lateralmost border of the RLl, while the upper visual field is represented nearer the medial border of RLl. One CTB injection within the SC failed to label terminals within RLl (Fig. 2.12). It is unclear exactly why this was the case because the injection depth was similar to other cases. No retrogradely labeled cells were present within RLl in any of the cases.

All SC injections labeled axon terminals within RLm. Projections to RLm from the SC also appear to be retinotopically organized. Injections made within the upper visual field representation of the SC labeled terminals in more medial aspects of RLm, while lower field SC injections produced terminal label in more lateral aspects of RLm (compare Fig. 2.11, 2.8, 2.10). Thus, it is likely that the upper visual field is represented medial to the lower visual field in RLm. Other aspects of the topographic organization within RLm were unclear. Much like RLl, no retrogradely labeled cells were present within RLm.

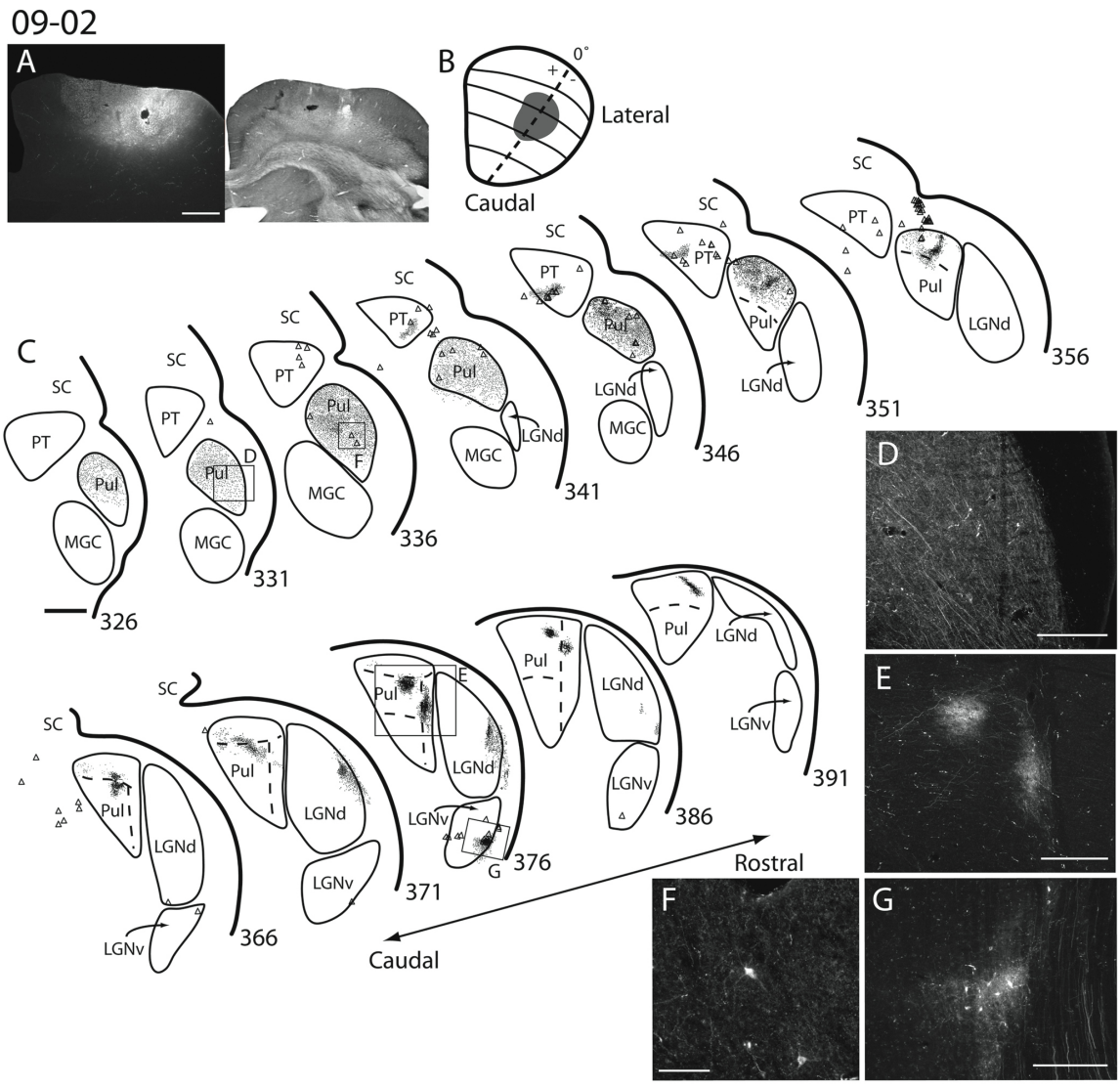


Figure 2.9. Superior colliculus (SC) connections with visual thalamus in squirrel 09-02. ***A****: Photomicrograph of fluoro-ruby (FR) injection site on the left and an image of the adjacent cytochrome oxidase (CO) section on the right.* ***B****: Dorsal view of the extent and estimated retinotopic location of the FR injection site.* ***C****: Coronal sections of thalamus with the locations of axon terminals (small black dots) and retrogradely labeled cell bodies (black triangles). Sections are arranged in a caudal (top left) to rostral (bottom right) direction.* ***D,E,G*** *are high-power images of axon terminal label within the pulvinar and ventral lateral geniculate nucleus.* ***F*** *shows labeled cell bodies and axon terminals within the caudal pulvinar from section 336 in B. D corresponds to the boxed area in section 331; E corresponds to the large boxed area in section 376; G corresponds to the smaller boxed area in section 376. Scale bars . 1 mm in A,C; 250* ***l****m in D; 0.5 mm in E; 100 μm in F; 0.25 mm in G.*

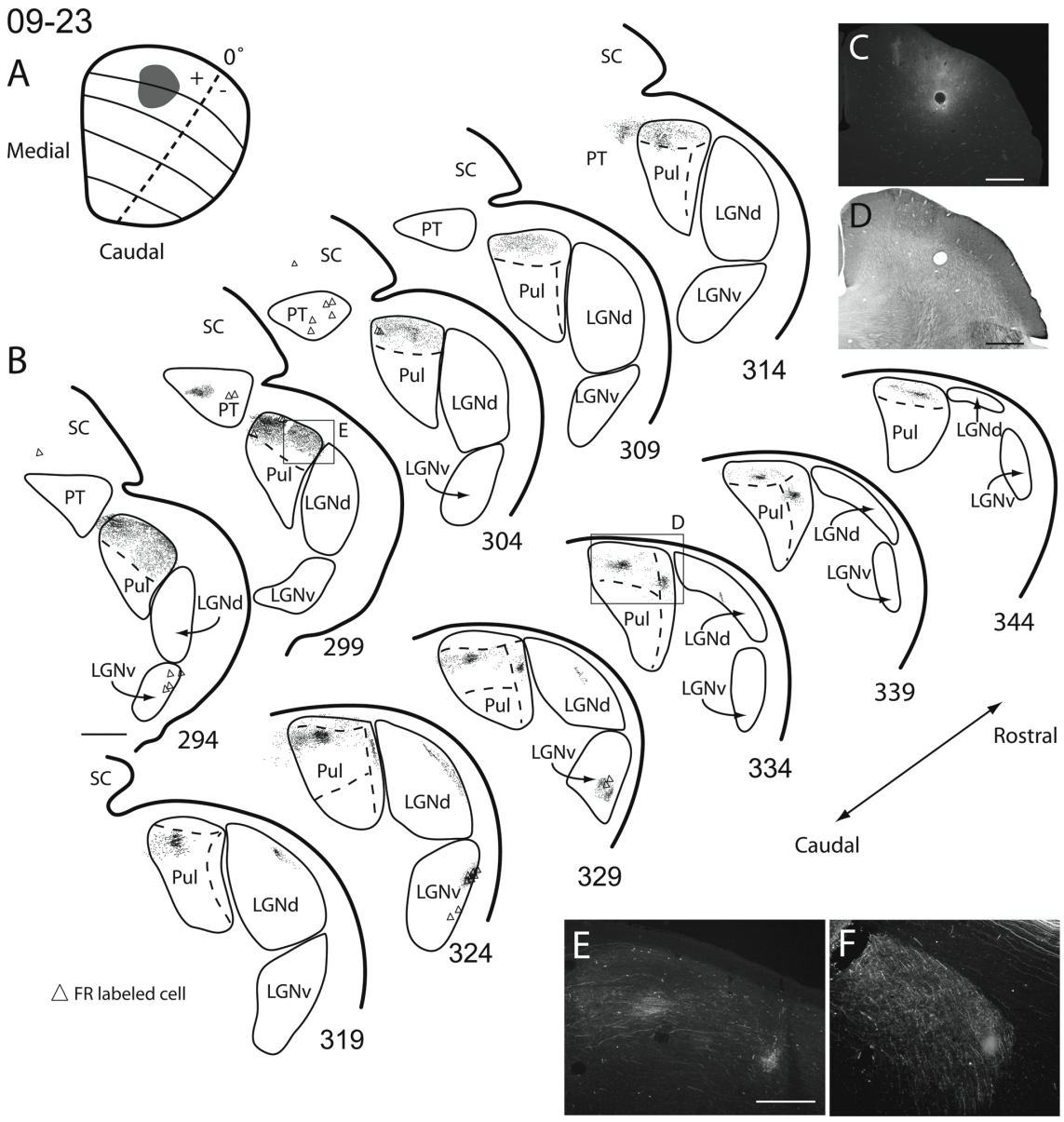


Figure 2.10. Superior colliculus (SC) connections with visual thalamus in squirrel 09-23. ***A****: The extent and estimated retinotopic location of the fluoro-ruby (FR) injection site are indicated on a reconstructed dorsal view of the SC.* ***B****: Coronal sections of thalamus with the reconstructed locations of axon terminals (dots) and labeled cells (triangles) within each section.* ***C****: High-power photomicrograph of the FR injection site.* ***D****: Photomicrograph of the adjacent cytochrome oxidase (CO) section to C.* ***E****: High-power image of the axon terminals within the box in section 334 shown in B.* ***F****: High-power image of the axon terminals within the box in section 299 in B. Scale bars . 1 mm in B–D; 0.5 mm in E; 250 μm for F.*

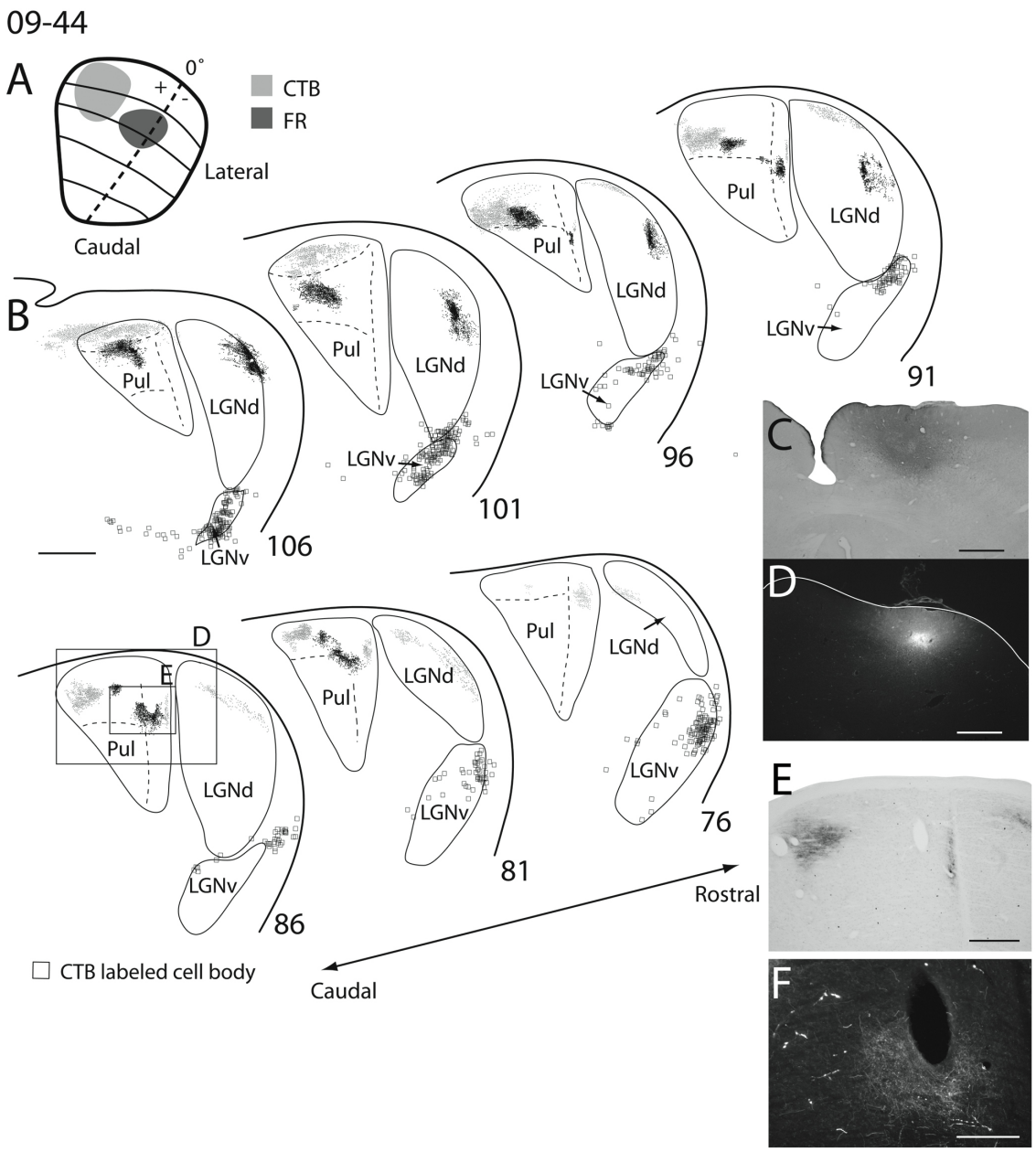


Figure 2.11. Superior colliculus (SC) connections with visual thalamus in squirrel 09-44. ***A****: Reconstructed dorsal view of the SC with the extent and location of the fluoro-ruby (FR; dark gray) and choleratoxin subunit B (CTB; light gray) injection sites.* ***B****: Coronal sections of thalamus arranged in a caudal, top left, to rostral, bottom right, manner, with the location of labeled axon terminals for CTB (light gray dots) and FR (dark gray dots) as well as retrogradely labeled CTB (squares) and FR (triangles) in each section.* ***C****: Magnified photomicrographs of the CTB injection site in a coronal section.* ***D****: Magnified photomicrograph of the FR injection site in a coronal section.* ***E****: Highpower image of CTB anterograde label within the large box in section 86.* ***F****: High-power image of FR anterograde label within the small box in section 86. Scale bars . 1 mm in B–D; 0.5 mm in E; 250 μm F.*

2.4.2.1.3. Rostral medial pulvinar

Injections within the SC in all of our cases failed to show any labeled cells or axon terminals within the rostral medial pulvinar. This is consistent with previous findings (Robson and Hall, 1977).

2.4.2.2. SC connections with the dorsal and ventral lateral geniculate nuclei

2.4.2.2.1. Dorsal lateral geniculate nucleus

In our observations, the superior colliculus projects to the third layer of the LGNd in a topographic manner as described in previous reports (Kaas et al., 1972b; Robson and Hall, 1976). More rostral injections within the SC produced labeled terminals more dorsally, while more caudal injections resulted in labeled terminals ventrally (compare Fig. 2.11, and 2.12, with Fig. 2.8). These results are consistent with the known retinotopy of the LGNd (Kaas et al., 1972b). As the LGNd is not known to project to the SC, no retrogradely labeled cells were found in the LGNd after SC injections.

2.4.2.2.1. Ventral lateral geniculate nucleus

Injections within the SC resulted in many retrogradely labeled cells within the LGNv. Anterogradely labeled terminals were also found within the LGNv (Fig. 2.9, 2.10). Similar results have been reported previously (Robson and Hall, 1977; Lugo-Garcia and Kicliter, 1988). Most labeled cell bodies and axon terminals were found within the lateral-most layer of the LGNv and the IGL (Fig. 2.9, 2.10, and 2.11).

2.4.2.3. Superior colliculus connections with other subcortical nuclei

2.4.2.3.1. Pretectum

SC injections resulted in both anterogradely labeled axon terminals as well as retrogradely labeled cells within nuclei of the ipsilateral pretectum (Fig. 2.8, 2.9, and 2.10). Most of the illustrated label was in the nucleus of the optic tract (NOT) and

09-50

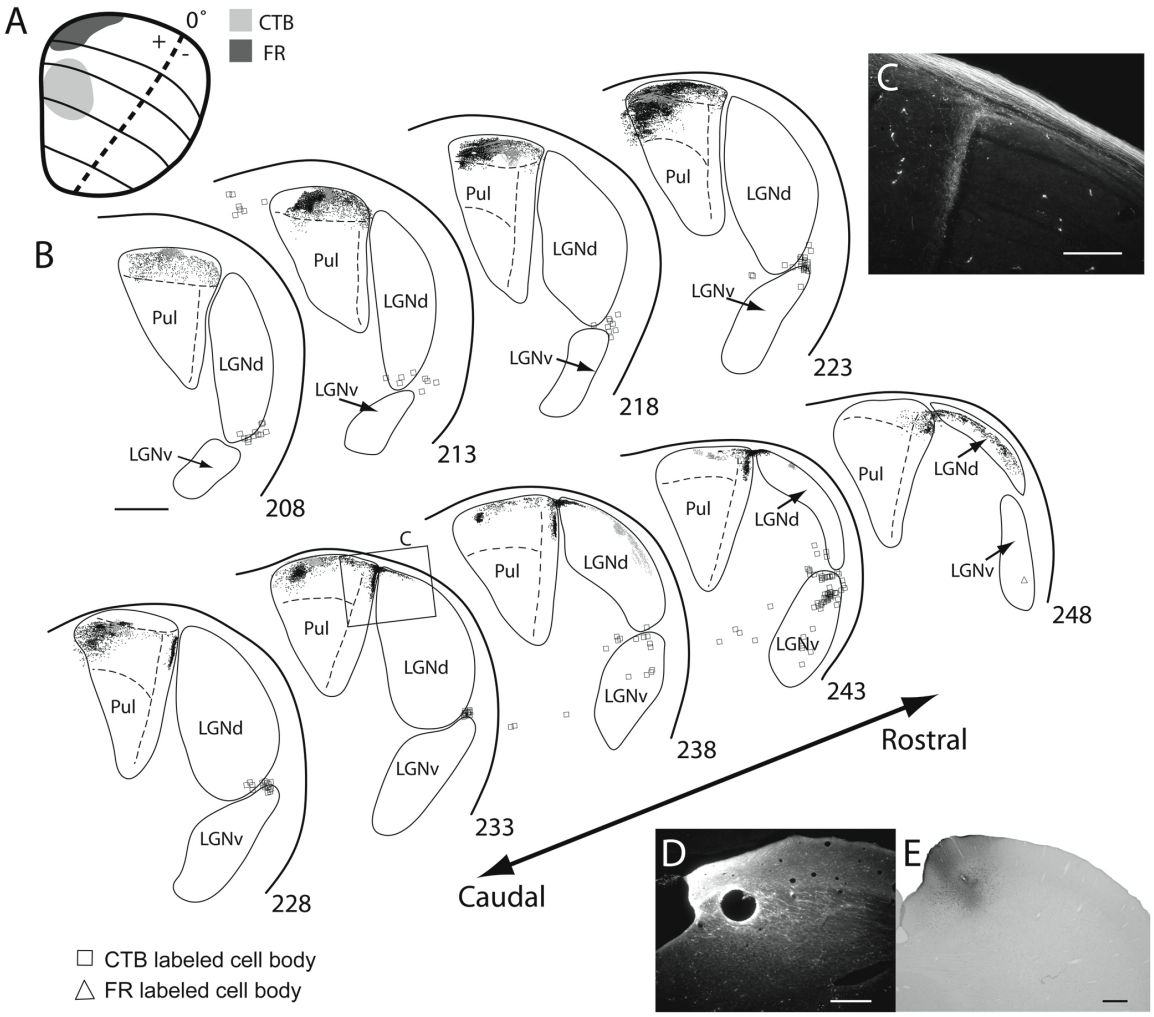


Figure. 2.12. Superior colliculus (SC) connections with visual thalamus in squirrel 09-50. ***A****: Dorsal view of the SC with the location and extent of choleratoxin subunit B (CTB; light gray) and fluoro-ruby (FR; dark gray) injection sites.* ***B****: Coronal sections of thalamus arranged in a caudal (top left) to rostral (bottom right) progression. The location of CTB-labeled axon terminals (dots) and CTB-labeled cells (squares), as well as FR labeled axon terminals (dots) and FR labeled cells (triangles) are presented for each section.* ***C****: Magnified photomicrograph of FR label in section 233 of B.* ***D****: High-power images of the FR injection site in coronal section.* ***E****: Photomicrograph of CTB injection site in coronal sections. Scale bars . 1 mm in B; 250* ***l****m in C; 0.5 mm in D,E.*

possibly the posterior pretectal nucleus (PPN) as described by Major et al (2003). Injections into the lateral SC resulted in label more laterally within PT, while injections into medial SC resulted in label more medially within PT (not shown). Connections of the pretectum with the SC have been described previously (Robson and Hall, 1977).

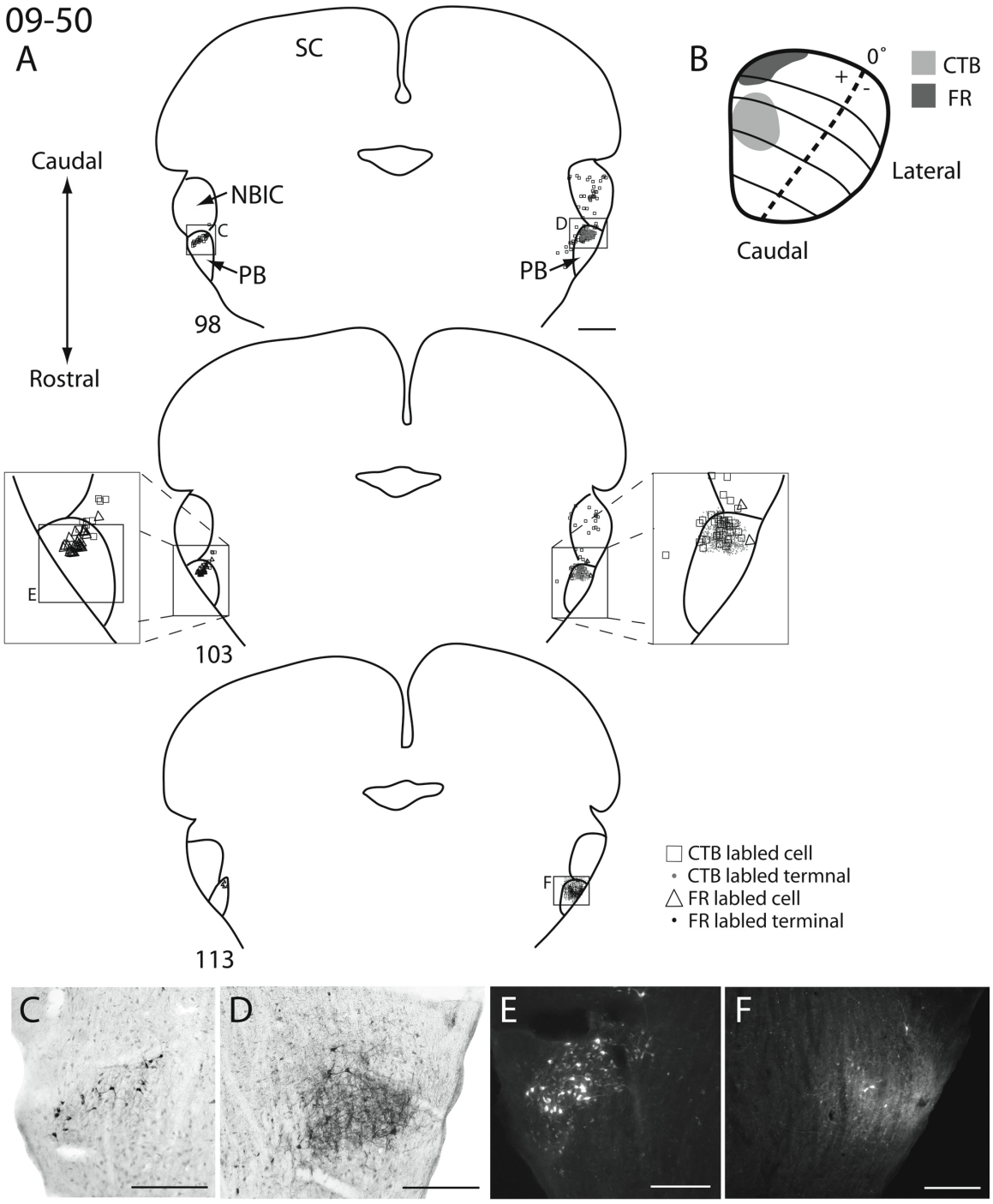
2.4.2.3.1. Nucleus of the brachium of the inferior colliculus

Retrogradely labeled cell bodies were present within the ipsilateral NBIC (Fig. 2.13). Anterogradely labeled axon terminals were not observed.

2.4.2.3.2. Parabigeminal nucleus

Both retrogradely labeled cell bodies and anterogradely labeled axon terminals were present within the ipsilateral parabigeminal (PB) nucleus, while only retrogradely labeled cells were found within the contralateral PB nucleus (Fig. 2.13). Injections within the rostral SC labeled axon terminals and cells more rostrally within PB, while caudal SC injections labeled axon terminals and cells more caudally (Fig. 2.13).

Figure 2.13. Superior colliculus (SC) connections with the parabigeminal nucleus in squirrel 09-50. ***A****: Coronal sections of the midbrain arranged in a caudal (top) to rostral (bottom) progession.* ***B****: Dorsal view of the SC with the location and extent of CTB (light gray) and FR (dark gray) injections sites.* ***C****: Magnified photomicrograph of CTB label in the contralateral parabigeminal nucleus in section 98.* ***D****: Magnified photomicrograph of CTB label in the ipsilateral parabigeminal nucleus in section 98.* ***E****: Magnified photomicrograph of FR label in the contralateral parabigeminal nucleus in section 103.* ***F****: Magnified photomicrograph of FR label in the ipsilateral parabigeminal nucleus in section 113. Scale bars . 1 mm in A; 0.25 mm in C,D,F; 0.1 mm in E.*


09-50
A
SC
Caudal
Rostral
NBIC
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98
D
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B
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CTB
FR
Lateral
Caudal
E
103
F
113
CTB labled cell
CTB labled termnal
FR labled cell
FR labled terminal
C
D
E
F

2.5. DISCUSSION

The current study focused on analyzing connections between the superior colliculus and pulvinar in grey squirrels. Our results show that the superior colliculus of grey squirrels sends projections to three architectonically distinct subdivisions of the pulvinar. The SC projects diffusely to the caudal division of the pulvinar, as well as sending two more focused projections to two subdivisions, medial RL (RLm) and lateral RL (RLl) within the previously defined RL division (Robson and Hall, 1977). These two newly designated RL subdivisions can be differentiated from one another using AChE and Nissl staining techniques (Fig. 2.3 and 2.5). SC projections to each of the two subdivisions of RL appear to be topographically organized with the upper visual field represented medially within RLm and lower visual field represented laterally (see Fig. 2.8 to 2.12). Within RLl, the lower visual field is represented medially, and the upper visual field is represented laterally (compare Fig. 2.11, with Fig. 2.9 and 2.12). Additionally, more rostral injections (representing frontal vision) project to more dorsal locations within the lateral RLl, while more caudal injections (representing the peripheral visual field) project ventrally within RLl.

Connections between the SC and the caudal division of the pulvinar are reciprocal, with both anterogradely labeled axon terminals and a sparse distribution of retrogradely labeled cells within this division after SC injections. Additionally, we provide evidence that there may be a crude representation of the visual field within the caudal pulvinar (compare Fig. 2.8 with Fig. 2.9 and 2.10). Recent evidence suggests that the 'diffuse' SC projections to the caudal pulvinar of tree shrews also have a connectional topography (Chomsung et al., 2008). As in previous studies, we did not find any

connections between the SC and the RM pulvinar (Robson and Hall, 1977; Lugo-Garcia and Kicliter, 1988).

We observed SC projections to dorsal lateral geniculate nucleus (LGNd), the ventral lateral geniculate nucleus (LGNv), the pretectum (PT), the nucleus of the brachium of the inferior colliculus (NBIC), and the parabigeminal nucleus (PB). All of these nuclei, except LGNd and the NBIC, were reciprocally connected with the SC. Projections from the SC to the LGNd were confined to layer 3, which is consistent with previous findings (Robson and Hall, 1976; Robson and Hall, 1977); however, Harting et al. (1991) suggest that the SC projects to layer 1 as well. Superior colliculus projections to the LGNd were topographically organized, with rostral SC injections producing labeled terminals more dorsally, and more caudal injections resulting in labeled terminals ventrally. These results are consistent with previous anatomical and physiological studies of LGNd connections and retinotopy in grey squirrels (Kaas et al., 1972a; Kaas et al., 1972b; Robson and Hall, 1976).

Retrogradely labeled cells, as well as labeled axon terminals, were observed within the LGNv after SC injections. Both retrogradely labeled cells and terminals were also observed within the PT and after SC injections. Similar observations have been reported for other mammals (Weber and Harting, 1980) and squirrels (Robson and Hall, 1977; Lugo-Garcia and Kicliter, 1988). Retrogradely labeled cells were observed in the ipsilateral nucleus of the brachium of the inferior colliculus (Fig. 2.13). In cats (Kudo et al., 1984), this nucleus has been shown to project to the intermediate layers of the SC. Retrogradely labeled cells were also present in both the ipsi- and contralateral PB (Fig. 2.13), while anterograde label was found only within the ipsilateral PB, which is

consistent with previous reports in grey squirrels (Holcombe and Hall, 1981), rats (Taylor et al., 1986), cats (Graybiel, 1978; Sherk, 1979; Roldán et al., 1983), and monkeys (Harting et al., 1980; Baizer et al., 1991). In the current study we were able to demonstrate that the projections to and from the PB are retinotopically organized with more rostral SC injections producing label in more rostral PB, and more caudal injections producing label in more caudal regions of the PB (Fig. 2.13). Evidence for such a map has been reported elsewhere (Sherk, 1979; Roldán et al., 1983; Baizer et al., 1991).

Finally, our architectonic analysis of the layers within the SC suggests that the SGI has three architectonically distinct sublayers. Thus the SC of grey squirrels has a more complex and distinct laminar pattern than previously reported in squirrels (May, 2006), but three sublayers of SGI have been recognized in unstained tissue samples of SC of rats (Helms et al., 2003). Such sublayers of SGI have not been noticed in the SC of tree shrews, which also have an enlarged and distinctively laminated SC (Harting et al., 1973b).

2.5.1. Relation to previous studies of SC projections to the puvlinar in squirrels

Results from this study confirm and expand the results from previous studies of grey squirrel pulvinar organization. In the first relevant study, Abplanalp (1970) described the pulvinar as a homogeneous structure with overlapping SC and striate projection zones. A later study by Kaas et al. (1972b) suggested that there are divisions within the pulvinar, as the caudal region of the pulvinar projected to the temporal intermediate (Ti) and temporal posterior (Tp) areas visual cortex, and the rostral pulvinar

projected to occipital areas 18 and 19. At that time, however, it was not clear if these connectional differences corresponded to architectonic divisions of the pulvinar. Subsequently, Robson and Hall (1977) divided the pulvinar of grey squirrels into three divisions based on differences in cytoarchitecture and connections. The caudal division (C) received diffuse projections from the SC and the rostral lateral division (RL) received "patchy", focused projections from the SC. The rostral medial division (RM) did not receive projections from the SC. Robson and Hall (1977) also found that rostral SC projected to more rostral locations within RL, and caudal SC projected to more caudal locations.

A major finding of the current study is the presence of two topographically organized projections within the RL division described by Robson and Hall (1977). Not only are there two topographically organized SC projection fields within RL, but we also found cytoarchitectural differences between the two fields (Fig. 2.3 and 2.5) suggesting that RL is comprised of two anatomically and functionally distinct divisions, RLl and RLm.

In the present study we were able to demonstrate a central-to-peripheral visual field retinotopy along a dorsal/ventral axis within RLl, and an upper-to-lower field retinotopy along the medial/lateral axis for both RLl and RLm (Fig. 2.14). However, the results from the present experiments did not clearly verify the existence of a rostral/caudal retinotopic organization in RL as described by Robson and Hall (1977), possibly because our results indicate that SC projections terminate in two locations in RL: the RLl and RLm. While Robson and Hall (1977) concluded that their lesions and injections were not varied enough to determine the topographic organization within RL

outside of the rostral/caudal domain, topographic patterns of connections consistent with present results are discernable in their results (Fig. 2.5, and Fig. 2.7 of Robson and Hall, 1977).

An additional observation in the present study was the presence of retrogradely labeled cells within the caudal division of the pulvinar complex. While the SC projects to the dorsal thalamus in all mammals, nuclei of the dorsal thalamus are not known to project to the SC (Jones, 2007). Yet, our observation within grey squirrels is not novel, as it has also been reported for the caudal pulvinar in ground squirrels (Lugo-Garcia and Kicliter, 1988). Other characteristics of the caudal pulvinar include strong CO, AChE and VGluT2 staining (Fig. 2.3). These characteristics helped distinguish the caudal pulvinar from rostral pulvinar divisions.

2.5.2. Cotical connections of grey squirrel pulvinar

Patterns of cortical connections also distinguish the main divisions of the pulvinar complex in squirrels (see Fig. 2.14D for proposed subdivisions of cortex in squirrels). Kaas et al., (1972b) showed that areas 18 and 19 receive projections from the rostral pulvinar, while the caudal pulvinar sends input to temporal areas such as Ti and Tp. To add to this, Robson and Hall (1977) found that RM receives inputs from areas 17, 18, and 19 and sends projections to area 19. RL projects to area 18, and the caudal pulvinar projects to areas within temporal cortex. However, recent studies using injections restricted to Ti failed to produce label within the pulvinar (see Fig. 9, Wong et al., 2008). This observation is consistent with the results of Robson and Hall (see Figs. 12, 15, and 17 of Robson and Hall 1977). Wong et al, (2008) also described an area medial to Tp

called the temporal mediodorsal area (Tm), which was previously described as part of area 19 (19p) (Kaas et al., 1972b; Robson and Hall, 1977). When retrograde tracer injections involved Tm, labeled cells were found in the location of RLl (see Fig. 2.9 and 2.13 of Wong et al., 2008). When injections were placed in Tp, labeled cells were found mainly within the caudal pulvinar (Wong et al., 2008). Overall, given our current subdivision of RL, we propose that the caudal pulvinar has reciprocal connections with area Tp, RLl projects to Tm, RLm projects to area 18, and RM projects to area 19 and receives projections from areas 17, 18, and 19 (Fig. 2.14). These nuclei may have other cortical connections that have not been studied. As area Tm has direction selective cells (Paolini and Sereno, 1989), RLl may relay direction selective information from the superior colliculus (Michael, 1972) to Tm.

2.5.3. The pulvinar complex of other rodents and other mammals

The superior colliculus likely projects to the caudal thalamus of all or nearly all mammals (Diamond, 1973; Jones, 2007; Kaas, 2007; Chomsung et al., 2008). This region of SC input, commonly included in a lateral posterior nucleus, makes up part or parts of the visual pulvinar. Other parts of the visual pulvinar without SC inputs have connections with visual cortex. Thus, the pulvinar complex has often been divided into nuclei with or without tectal input. Such subdivisions have been described in a variety of mammals including hedgehogs (Gould et al., 1978), rats (Mason and Groos, 1981; Takahashi, 1985), squirrels (Robson and Hall, 1977; Lugo-Garcia and Kicliter, 1988), tree shrews (Abplanalp, 1970; Harting et al., 1973a; Lyon and Kaas 2003b; Chomsung et al., 2008), cats (Graybiel, 1974; Berson and Graybiel, 1978), galagos (Glendenning, et al., 1975), and New World and Old World monkeys (Cusick et al., 1993; Stepniewska and Kaas,

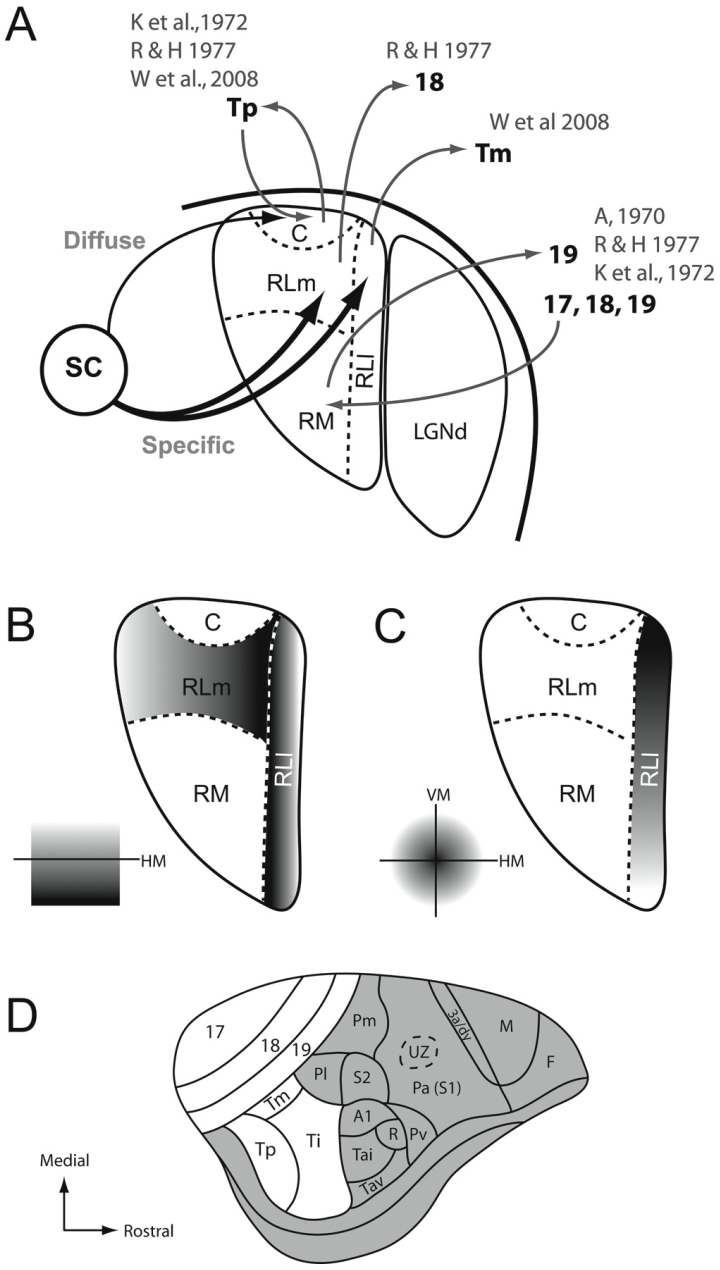

Figure 2.14. Current proposal of gray squirrel pulvinar organization. ***A****: Summary of connections between the superior colliculus and pulvinar complex as well as connections between the pulvinar complex and visual cortex in gray squirrels. A, 1970 is Abplanalp (1970); K et al., 1972b is Kaas et al. (1972b); R&H, 1977 is Robson and Hall (1977); W et al., 2008 is Wong et al. (2008).* ***B****: Topographic organization of RLl and RLm based on upper and lower field representations.* ***C****: Topographic organization within RLl based on central and peripheral visual field representations.* ***D****: Lateral view of the right hemisphere of a gray squirrel with occipital, and temporal visual areas highlighted in white (adapted from Wong and Kaas, 2008).*

1997; Stepniewska et al., 2000; Lyon et al., 2010). The tectofugal projections arise from superficial SC layers (grey squirrels; Robson and Hall 1977; tree shrews: Graham and Casagrande, 1980; squirrel monkeys: Huerta and Harting, 1983; macaques: Trojanowski and Jacobson, 1975; Benevento and Standage, 1983; Lyon et al., 2010). A common theme for most mammals is the presence of a caudal nucleus of the pulvinar that strongly expresses AChE, receives projections from the SC, and has connections with temporal visual areas (see Lyon and Kaas, 2003b, Chomsung et al., 2008 for review). However, species differences occur in the general organization of the pulvinar and number of divisions of the pulvinar beyond this strongly AChE staining SC projection zone.

In addition to squirrels, aspects of pulvinar (lateral posterior nucleus) organization have been studied in other rodents such as rats, hamsters, and degus. In rats, the pulvinar (the lateral posterior-pulvinar) has been divided into 4 nuclei (Takahashi, 1985) (Fig. 2.15D), including a rostral cortico-recipient zone and a caudal tecto-recipient zone (Mason and Groos, 1981; Masterson and Bickford, 2009. Bilateral SC inputs to the caudal pulvinar of rats were first reported in a region that projects to temporal cortex (Mason and Groos, 1981). Takahashi (1985) subsequently reported that the SC projects to two different subdivisions of pulvinar complex, with bilateral inputs to lateralis posterior caudomedialis (lpcm), and focused ipsilateral inputs to caudal lateralis posterior pars lateralis (lplc). A third nucleus, lpl pars rostralis (lplr), did not receive SC projections but received cortical projections from striate and extrastriate cortex. The fourth nucleus, the lateralis posterior pars rostromedialis (lprm), did not receive projections from the SC, but received projections from striate cortex and cortex adjacent to striate cortex. On the basis of relative position and connections, lpcm likely corresponds to the caudal nucleus, C, of

squirrels and lprm of rats to the RM of squirrels. Other potential homologies are less certain.

In hamsters, Crain and Hall (1980) divided the pulvinar (LP nucleus) into three subdivisions based on differences in myelo- and cytoarchitecture, and connections with the SC and visual cortex. A caudal nucleus, LPc, received evenly distributed bilateral inputs from the SC, while a rostral lateral, LPrl, received more focused inputs from the SC. A rostral medial nucleus, LPrm, did not receive inputs from the SC, much like RM in grey squirrels. Later studies by Ling and colleagues (1997) split the LP of hamsters into four divisions based on architecture and connections, and the borders determined in the Crain and Hall study were slightly modified (Fig. 15C). The terminology for these subdivisions was changed in order to associate the subdivisions with respect to their position relative to the optic tract. Therefore, LPrm of Crain and Hall (1980) was renamed the deep division (LP-d) and LPrl was renamed the superficial division (LP-s). The fourth subdivision was located along the medial rostral aspect of LP and was named the medial subdivision (LP-m). Additionally, Ling et al., (1997) proposed that the SC projects to all divisions of LP. Retinotopic organizations within the subdivisions of LP have not been described in hamsters, and there is little understanding of cortical connections. However, injections of retrograde tracers into area 17 of hamsters labeled a few cells primarily within the rostral dorsal region of LP, while injections involving laterally adjacent cortical visual areas labeled cells throughout the full extent of LP (Dürsteler et al., 1979). As these connections were determined prior to the identification of divisions of LP described by Crain and Hall (1980) or Ling et al., (1997), the divisions of the pulvinar that project to occipital cortex are uncertain. Overall, the LPc nucleus of

hamsters appears to correspond to the caudal nucleus of the pulvinar of grey squirrels, but other correspondences are uncertain.

Octodon Degus, or degu, is a diurnal, ground dwelling rodent found in Chile that has evolved independently from North American rodents for over 40 millions years (Chaline, 1977). The pulvinar complex has been divided by Kuljis and Fernandez (1982) into three subnuclei: a caudal division with projections from the SC, a rostral medial division (RM) with connections from the SC, and a rostral lateral (RL) division, which is void of projections from the SC (Fig. 2.15B). Thus, the RM and RL connections appear to be opposite of those found in grey squirrels. SC projections to the caudal division and RM are topographically organized (Kuljis and Fernandez, 1982). Both RM and RL project to cortex lateral to primary visual cortex, V1 (or area 17), but RL may project largely to cortex adjacent to V1, and RM more to more lateral cortex, and the caudal pulvinar projects to temporal cortex. As in other rodents, the degu has a caudal nucleus that projects to temporal cortex. The RM nucleus of degu appears to correspond to the RLm nucleus of squirrels, while the RL nucleus corresponds to RM of squirrels. This difference in locations could reflect a simple rotation and the absence of evidence for a RLl.

In summary, there appears to be a conserved organization within the pulvinar/lateral posterior complex in rodents with diffuse, bilateral SC projections to a caudal nucleus, and at least one other more focused projection within the rostral aspect of the nucleus. Additionally, in most rodents there is a portion of the pulvinar/LP that is void of SC projections (though see Ling et al., 1997), but has inputs from striate cortex.

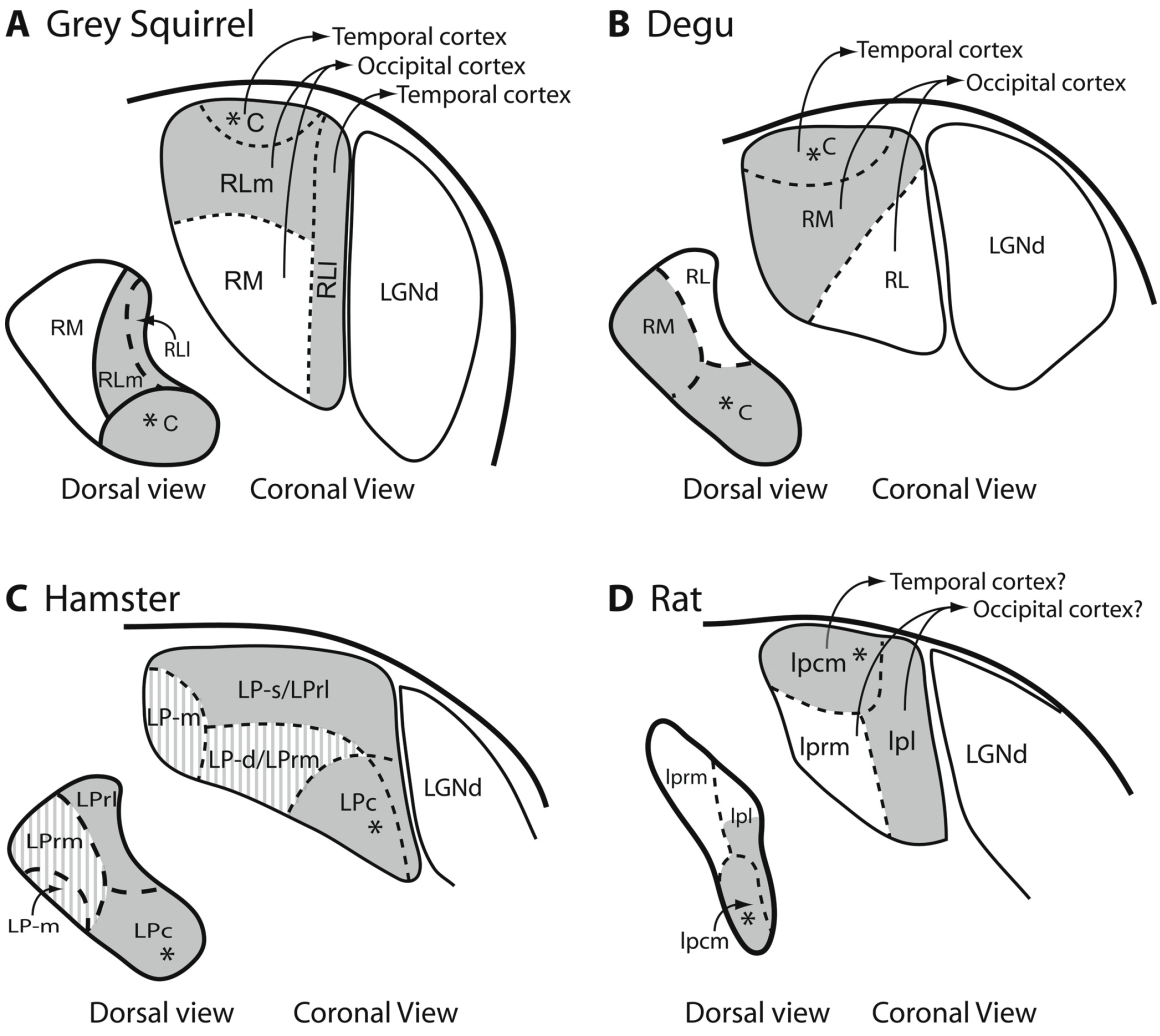

*Figure 2.15. Possible pulvinar/lateral posterior complex organization schemes for gray squirrel (**A**), degu (**B**), hamster (**C**), and rat (**D**). Subdivisions of the pulvinar that receive SC projections are highlighted in gray. Asterisk symbols represent bilateral SC input. Information for A is based on descriptions from Robson and Hall (1977), Wong et al. (2008), and the current study. B is based on descriptions from Kuljis and Femandez (1982). C is based on descriptions from Crain and Hall (1980) and Ling et al. (1997). The gray lined areas represent discrepancies between Crain and Hall (1980) and Ling et al. (1997) with respect to SC projections. D is based on descriptions of Takahashi (1985).*

While grey squirrels have three distinctly different SC projection zones within the pulvinar, other rodents may have only two.

Squirrels are more distant and tree shrews are closer relatives of primates in the Euarchontoglire clade. As tree shrews and squirrels have evolved enlarged, diurnal visual

systems, these visual systems share a number of similarities due to their genetic relationship and to convergent evolution (see Kaas et al., 2002 for review). Although an enlargement of the pulvinar complex occurred in lines leading to both present day squirrels and tree shrews, the enlarged complex in each mammal has been subdivided in somewhat different ways. The pulvinar complex of tree shrews has four subdivisions that can be differentiated based on architecture and connections with the SC and cortex (Lyon et al., 2003a;b). Of these four, two subdivisions are known to receive SC projections, the dorsal subdivision, Pd, and the central subdivision, Pc (Luppino et al., 1988; Lyon et al 2003b; Chomsung et al., 2008). Similar to the caudal division of the grey squirrel, Pd stains darkly for AChE receives diffuse projections from the SC and sends projections to temporal cortical areas including Tp (Luppino et al., 1988; Lyon et al., 2003a; Chomsung et al., 2008; Chomsung et al., 2010). Pc shares similarities with RLl of squirrels, as Pc receives retinotopically organized projections from the SC and projects to temporal cortex including dorsal temporal cortex (Luppino et al., 1988; Chomsung et al., 2008; Chomsung et al., 2010). Another subdivision of the tree shrew pulvinar, the ventral pulvinar (Pv) does not receive projections from the SC, but does have connections with occipital cortical areas (Luppino et al., 1988; Lyon et al., 2003b). Thus, Pd, Pc, and Pv are likely homologous with the caudal nucleus (C), RLl, and RM of squirrels, respectively. Tree shrews also have a posterior pulvinar nucleus, Pp, which does not appear to correspond to any part of the pulvinar complex in squirrels, and tree shrews do not appear to have a homologue of the squirrel RLl nucleus.

In primates, the traditional divisions of the pulvinar include the anterior, medial, lateral, and inferior nuclei (Kaas & Huerta, 1988). The inferior pulvinar and lateral

pulvinar have been further divided into nuclei that are involved in vision, while the medial and anterior pulvinar share connections with multisensory, sensorimotor, and somatosensory areas (Pons and Kaas, 1985; Stepniewska, 2004; Gharbawie et al., 2010). In prosimian galagos, the inferior pulvinar receives the bulk of SC projections and it appears that there are two projection zones within the inferior pulvinar (see Fig. 12 of Wong et al., 2009). The posterior aspect of the inferior pulvinar of galagos receives SC projections and in turn projects to temporal cortex (Glendenning et al., 1975). Thus, parts of the inferior pulvinar resemble the caudal pulvinar and RLl of grey squirrels. In New and Old World monkeys the inferior pulvinar has at least three divisions that receive SC projections. Both the posterior inferior pulvinar (PIp) and the caudal medial inferior pulvinar (PIcm) receive dense projections from the SC. As PIp stains darkly for AChE (Stepniewska, 1997) and sends projections to temporal cortical visual areas (Stepniewska et al., 2000), it clearly resembles the caudal pulvinar of grey squirrels. PIcm, stains lightly for AChE and projects to the temporal cortical visual areas (Lin and Kaas 1979; Huerta and Harting, 1983; Stepniewska et al., 2000). Thus, the connectional properties of PIcm are similar to RLl of grey squirrels. A third division, the caudal lateral inferior pulvinar (PIcl), stains moderately dark for AChE and projects to occipital visual areas (Stepniewska et al., 1997; Stepniewska et al., 2000; Kaas and Lyon, 2007), similar to RLm of grey squirrels. The lateral pulvinar of New and Old World monkeys receives SC projections (Harting et al., 1978; Harting et al., 1980; Huerta and Harting, 1983) but they are slight and not always obvious (Partlow et al., 1977; Stepniewska et al., 1999). As the lateral pulvinar has connections within occipital visual areas (Kaas and Lyon, 2007), the lateral pulvinar in monkeys resembles RM of grey squirrels, which has connections with

occipital cortex and no obvious SC inputs. Overall, as many as 3 or 4 of the nuclei of the pulvinar in primates may have homologues in the pulvinar of squirrels and other rodents, as well as in tree shrews (see Lyon et al., 2003b for review).

In summary, most mammals appear to have a nucleus in the pulvinar that is similar to the caudal pulvinar of grey squirrels. Homologues between other pulvinar nuclei of squirrels and other rodents, as well as tree shrews and primates, are suggested. With further evidence, it would be useful to employ a consistent terminology.

2.5.4. What does the pulvinar do?

A number of investigators have suggested that the parts of the pulvinar with SC input act as an extrageniculate relay of visual information from the retina to cortex (Snyder and Diamond, 1969; Diamond, 1973; Chalupa, 1991). The types of SC cells projecting to the pulvinar originate from the lower stratum griseum superficiale (Robson and Hall, 1977). Recordings from these cells in tree shrews (Albano et al., 1978) and ground squirrels (Michael, 1972; Major et al., 2000) indicate that they respond best to large moving stimuli within the visual field. Thus, this pathway may provide information about movement within the visual field in its relay to visual cortex. Each of the subdivisions within the grey squirrel pulvinar projects to areas of cortex that respond with evoked potentials to visual stimulation (Hall et al., 1971). Given the lack of a detailed retinotopy, the caudal division of the pulvinar may provide motion information from direction selective motion cells in the SC (Michael, 1972; Major et al., 2000) to cortical area Tp, while the retinotopic divisions, RLm and RLl, may provide information about motion and location to areas 18 and Tm. In squirrels, many visual abilities, such as visual

pattern discrimination, are preserved after large lesions of V1 when extrastriate cortex remains intact (Wagor, 1978; Levey, 1973). Similar findings have been reported in tree shrews (Snyder and Diamond, 1968) and rats (Lweellyn et al., 1969; Mize et al., 1971). Additionally, squirrels with both striate and extrastriate cortex ablations lose the ability to perform the visual discrimination tasks they could perform after V1 lesions alone (Wagor, 1978). Given that the LGNd projects exclusively or nearly exclusively to primary visual cortex in grey squirrels, while the pulvinar provides information to extrastriate visual cortex (Kaas et al., 1972b), the alternative extrageniculate pathway through the SC and pulvinar likely provides the information needed for some visual discrimination tasks (Wagor, 1978). As squirrels retain some pattern vision after the removal of areas 17, 18, and 19; pulvinar projections to Tm and Tp may maintain this pattern vision.

2.6. ABBREVIATIONS

17	Area 17
18	Area 18
19	Area 19
A1	Primary auditory cortex
C	Caudal pulvinar
F	Frontal area
IGL	Intergeniculate leaflet
LGNd	Dorsal lateral geniculate nucleus
LGNv	Ventral lateral geniculate nucleus
LP	Lateral posterior nucleus
M	Primary motor cortex
MGC	Medial geniculate complex
NBIC	Nucleus of the brachium of the inferior colliculus
NOT	Nucleus of the optic tract
OT	Optic tract
PB	Parabigeminal nucleus
Pl	Parietal lateral area
Pm	Parietal medial area
Pul	Pulvinar
PT	Pretectum
Pv	Parietal ventral area
R	Rostral auditory area
RLl	Rostral lateral lateral pulvinar
RLm	Rostral lateral medial pulvinar
RM	Rostral medial pulvinar
S2	Secondary somatosensory cortex
SC	Superior colliculus
Tai	Temporal anterior intermediate area
Tav	Temporal anterior ventral area
Ti	Temporal intermediate area
Tm	Temporal mediodorsal area
Tp	Temporal posterior area
UZ	Unresponsive zone
V1	Primary visual area
V2	Secondary visual area
V3	Third visual area

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CHAPTER 3

SUBCORTICAL CONNECTIONS OF THE SUPERIOR COLLICULUS IN PROSIMIAN GALAGOS (*OTOLEMUR GARNETTII*[2])

3.1. ABSTRACT

An understanding of the organization of the pulvinar complex in prosimian primates has been somewhat elusive due to the lack of clear architectonic divisions. In the current study, we revealed features of the organization of the pulvinar complex in galagos by examining superior colliculus (SC) projections to this structure and comparing them with staining patterns of the vesicular glutamate transporter, VGLUT2. Cholera toxin subunit B (CTB), fluroruby (FR) and wheat germ agglutinin conjugated with horseradish peroxidase (WGA-HRP) were placed in topographically different locations within the SC. Our results showed multiple topographically organized patterns of projections from the SC to several divisions of the pulvinar complex. At least two topographically distributed projections were found within the lateral region of the pulvinar complex, and two less obvious topographical projection patterns were found within the caudomedial region, in zones that stain darkly for VGLUT2. Other subcortical projections of the SC were to the dorsal lateral geniculate nucleus, pretectal nuclei, and nucleus limitans, as well as reciprocal connections with the ventral lateral geniculate

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[2] This chapter is currently under review at the Journal of Comparative Neurology: Baldwin MKL, Balaram P, Kaas JH. Subcortical projections of the superior colliculus to the pulvinar in prosimian galagos (*Otolemur garnettii*) and VGLUT2 staining of the visual pulvinar: new subdivisions revealed.

nucleus, and parabigeminal nucleus. The results, in relation to recent observations in tree shrews and squirrels, suggest that parts of the organizational scheme of the pulvinar complex in primates are present in rodents and other mammals.

3.2. INTRODUCTION

The extrageniculate pathway, relaying visual information from the retina through the superior colliculus and pulvinar to visual cortex, has been found in all studied mammals (Harting et al., 1973; Diamond et al., 1976). This pathway provides an alternate route for visual information to reach extrastriate visual areas outside of the classical geniculate pathway projecting to striate cortex. Lesion studies suggest that the extrageniculate pathway is especially important for vision in some mammals such as tree shrews (Casagrande and Diamond, 1974) and squirrels (Levey, 1973; Diamond et al., 1973; Wagor, 1978). It may also be important for vision in prosimian galagos, as many visual abilities are maintained in galagos after striate cortex is removed (Marcotte and Ward, 1980, but see Atencio et al., 1975). In humans and Old World macaques, this pathway seems less important, but it may be involved in the unconscious aspects of vision termed blindsight (Poppel et al., 1973; Stoerig and Cowey, 2007; Tamietto et al., 2010).

Studying this extrageniculate pathway in galagos is of special interest because galagos, and other prosimian primates, have brains that appear to have changed the least from those of early primates (Radinsky, 1975; Preuss and Goldman-Rakic, 1991a, b; Preuss and Kaas 1993; Kaas, 2007a). As such, our understanding of the organization of the extrageniculate pathway in galagos can provide information on specializations and common features of primates, and perhaps suggest relationships to the pulvinar patterns of rodents and tree shrews, which are members of the Euarchontoglire clade with primates (Murphy et al., 2001; Kaas et al., 2002, 2005; Meredith et al., 2011).

In most species studied, dense projections from the superior colliculus terminate within the caudal aspect of the pulvinar complex. In squirrels and other rodents, tectal projections terminate diffusely within the caudal aspect of the pulvinar, while rostrally, the projections are more focused and topographic (Robson and Hall 1977; Crain and Hall, 1980; Kuljis and Fernandez, 1982; Ling et al., 1997; Takahashi et al., 1985; Baldwin et al., 2011). Tree shrews also have projections that terminate diffusely within the caudal aspect of the pulvinar, and additional more focused projections that terminate in more rostral locations (Luppino et al., 1998; Chomsung et al., 2008). In anthropoid primates, tectal projections terminate in two caudal locations within the inferior pulvinar and additional projections to more rostral and lateral positions have been observed in New World monkeys, but it is unclear if these more rostral and lateral projections are present in Old World monkeys (Stepniewska et al., 2000). Finally, previous studies in galagos have shown dense projections from the superior colliculus to the caudal aspect of the inferior pulvinar (Glendenning et al., 1975; Wong et al, 2008), with less dense projections extending into more rostrolateral locations (Diamond et al., 1992). An understanding of the differences and consistencies in the patterns of tectal projections across different members of the Euarchotoglire clade could provide insights into how the pulvinar, and the extrageniculate pathway through the pulvinar to cortex evolved.

Considerable progress has been made in recent years in understanding the organization of the pulvinar complex in anthropoid primates, where four nuclear divisions of the inferior pulvinar have been identified (Stepniewska et al., 1997, 2000; Stepniewska, 2004; Jones, 2007). Much less is known about the divisions of the pulvinar of galagos, mainly because of the lack of architectonic markers that distinguish between

divisions (Beck and Kaas, 1998; Wong et al., 2009). Therefore, it has been difficult to identify homologous pulvinar structures between galagos and other primates, and between primates and rodents (see Lyon et al, 2003 for review).

The current study had two main goals. The first was to determine the distribution pattern of the vesicular glutamate transporter, VGLUT2, within the pulvinar complex in galagos. This transporter has been shown to be a general marker of subcortical projections to the dorsal thalamus and sensory cortex (Herzog et al., 2001; Hackett et al., 2011; Balaram et al., 2011) and therefore could indicate where tectal terminations are located within the galago pulvinar (Balaram et al., 2011). The second goal was to directly determine the extent and organization of tectal projections to the pulvinar complex in galagos. As the projections from the superior colliculus to the different parts of the pulvinar have been described as topographic (retinotopic) or diffuse, we also wanted to evaluate this aspect of the tectal projection pattern in galagos. Our results show that VGLUT2 is a robust marker for some of the superior colliculus projections to the pulvinar complex, specifically projections to caudal subdivisions that project to higher order visual areas in temporal cortex. We also found at least two additional tectal projections to more rostral and lateral positions within the pulvinar complex that are not associated with VGLUT2 dense staining. Cortical projections arising from these pulvinar divisions are to occipital visual areas involved with early stages of cortical processing. Most importantly, our results identify homologous divisions of the pulvinar complex in prosimian and anthropoid primates, and suggest how features of the primate pulvinar evolved from non-primate ancestors.

3.3. MATERIALS AND METHODS

Tectopulvinar projections and histological architecture of the pulvinar were studied in adult 10 galagos (*Otolemur Garnettii*). All surgical procedures were conducted in accordance with an approved protocol by the Vanderbilt University Institutional Animal Care and Use Committee (IACUC) and followed the guidelines published by the National Institute of Health.

3.3.1. Surgical procedures

The methods in the present study are similar to those described elsewhere (Wong et al., 2009; Baldwin et al., 2011 and Baldwin and Kaas, 2012). Animals were initially anesthetized with an intramuscular injection of ketamine hydrochloride (120mg/kg) and anesthesia was maintained for the rest of the surgical procedures using 0.5 to 2% isoflurane delivered through a tracheal tube. Lidocaine was placed in both ears as well as along the midline of the scalp. An incision was then placed along the midline of the scalp and part of the left skull was exposed. A small craniotomy was made over the left parietal and occipital lobes. The dura was removed and medial portions of the parietal and occipital lobes were removed by aspiration to visualize the left superior colliculus. In 4 cases, injections of anatomical tracers were made within the left superior colliculus after cortical aspirations, while in an additional 4 cases, the medial aspect of the right hemisphere was retracted and injections were made along the medial wall of the right superior colliculus. An additional case was used for architectonic analysis only, and the final case was used for western blot analysis of the VGLUT2 antibody. Tracers were pressure injected at depths of 0.7 to 1.3mm from the surface of the superior colliculus using a Hamilton syringe fitted with a glass pipette beveled to a fine tip. The tracers used

in this experiment were 0.5-1 µl of cholera toxin ß-subunit (CTB: Molecular Probes Invitrogen, Carlsbad, CA; 10% in distilled water), 0.5 to 1 µl of fluoro-ruby (FR: Molecular Probes Invitrogen: 10% in phosphate buffer), and 0.1ul of wheat germ agglutinin conjugated with horseradish peroxidase (WGA-HRP: Sigma, St. Louis, MO; 2% in distilled water). Any tracer leakage during injections was removed with sterile saline flushes in order to prevent contamination of surrounding brain tissue. Injections lasted approximately 5 minutes to allow tracer to diffuse into the brain. Gelfoam was then placed into the aspirated region of cortex and a layer of gelfilm was placed over the brain within the region of the craniotomy. An artificial skullcap of dental cement covered the opening and was sealed to the skull. Surgical staples were used to close the incision site. Animals were then taken off anesthesia, and monitored during recovery. Once fully awake, galagos were given 0.3mg/kg of Buprenex analgesic and were returned to their home cage.

3.3.2. Histology

Five to seven days after surgery, animals were given a lethal dose of sodium pentobarbital (80mg/kg intravenously) and perfused with phosphate-buffer (PB; pH 7.4), followed by 2% paraformaldehyde in PB, and finally 2% paraformaldehyde in PB with 10% sucrose. After the brains were removed, the cortex was separated from underlying brain structures. The cortex from the intact hemisphere was flattened and used for another study (Baldwin and Kaas, 2012). The thalamus and brainstem were then placed in 30% sucrose solution for cryoprotection and stored at 4^{o}C for 20 to 48 hours.

The thalamus and brainstem were cut in the coronal plane using a freezing microtome at a thickness of 40 µm. The tissue was saved in 5 series. One to three series

were processed for anatomical tracers such as CTB using a histological procedures described in Baldwin et al., (2011), or WGA-HRP using procedures of Gibson et al. (1984), or were immediately mounted onto glass slides for fluorescent analysis of FR. Remaining series were processed for two to three of the following stains: cytochrome oxidase, CO (Wong-Wiley, 1979), acetylcholinesterase, AChE (Geneser-Jensen and Blackstand, 1971), vesicular glutamate transporter 2, VGLUT2 (mouse monoclonal anti-VGLUT2 from Millipore, Billerica, MA: 1:5000). For one case, two series of tissue were processed for VGLUT2 mRNA using previously described in situ hybridization techniques (Balaram et al., 2011). The CTB antibody was tested on galago brain tissue with no CTB injections and this control failed to label any cells or patches of axon terminals. The VGLUT antibody was tested against galago brain tissue using standard western blot techniques (Baldwin et al., 2011) and showed a single band at 56 kDa (Fig. 3.1), the known molecular weight of VGLUT2 (Aihara et al., 2000).

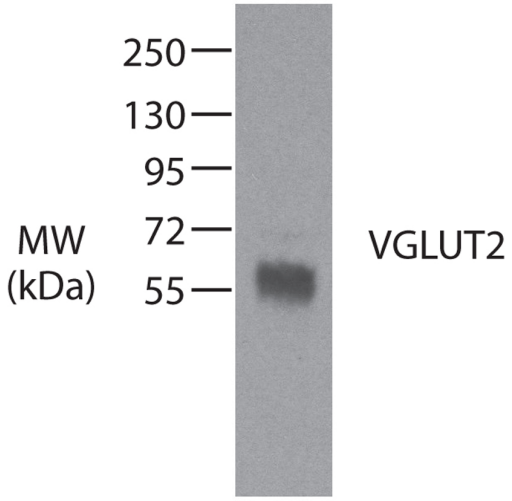


Figure 3.1. Western blot characterization of the VGLUT2 antibody in galago striate cortex. The antibody recognizes a 56 kDa protein, which is the known molecular weight of VGLUT2.

3.3.3. Data analysis

The locations of CTB, FR and WGA-HRP labeled axon terminals and cell bodies were plotted using an XY plotter (Neurolucida system: MicroBright, Williston, VT). Digital images of tissue sections were taken using a DXM1200F digital camera mounted to a Nikon E800S microscope (Nikon Inc., Melville, NY). Images were adjusted for brightness and contrast, but were otherwise not altered.

Labeled terminals and cell locations were related to thalamic architecture by matching plotted sections to adjacent brain sections using common blood vessels in Adobe Illustrator. Injection sites, anterogradely labeled terminals, and retrogradely labeled cell bodies in plotted sections were referenced to CO, VGLUT2, and AChE stained sections.

The locations of injection sites within the superior colliculus were placed in reference to dorsal views of the structure, after reconstructions from serial coronal sections. This dorsal reconstruction was then aligned with a previously determined retinotopic map of the contralateral visual hemifield (Lane et al., 1973). As in other primates, the upper visual quadrant is represented medially, the lower visual quadrant, laterally, peripheral vision caudally, and central vision, rostrally within the superior colliculus of galagos

3.4. RESULTS

In the present study, injections of fluororuby (FR), cholera toxin subunit B (CTB), or wheat germ agglutinin conjugated with horseradish peroxidase (WGA-HRP) were placed at various locations within the superior colliculus and the resulting patterns of anterograde and retrograde label were examined within subcortical visual structures. An emphasis was placed on investigating the superior colliculus projections to the pulvinar complex. Additionally, architectonic borders within and between subcortical structures were evaluated using cytochrome oxidase, acetylcholinesterase, and VGLUT2 preparations. The results indicate that there are multiple projections to the pulvinar complex from the superior colliculus. At least two projections terminate within domains of the posterior puvlinar that stain strongly for VGLUT2, while two or three projections terminate in more rostrolateral divisions of the pulvinar complex that stain weakly for VGLUT2. Here we briefly describe the architectonic characteristics used to identify thalamic and brain stem nuclei, including subdivisions within the pulvinar complex, followed by descriptions of the superior colliculus connection patterns within these subcortical visual structures.

3.4.1. Architecture of subcortical visual areas

3.4.1.1. Superior colliculus

Determining the laminar architecture of the superior colliculus was important for identifying the locations of tracer placements. The superior colliculus of galagos has seven main layers (Fig. 3.2A). The superficial layers consisting of the stratum zonale (SZ), the stratum griseum superficiale (SGS), the stratum opticum (SO) are visual in

function, while deeper layers are associated with integrating multisensory information and motor

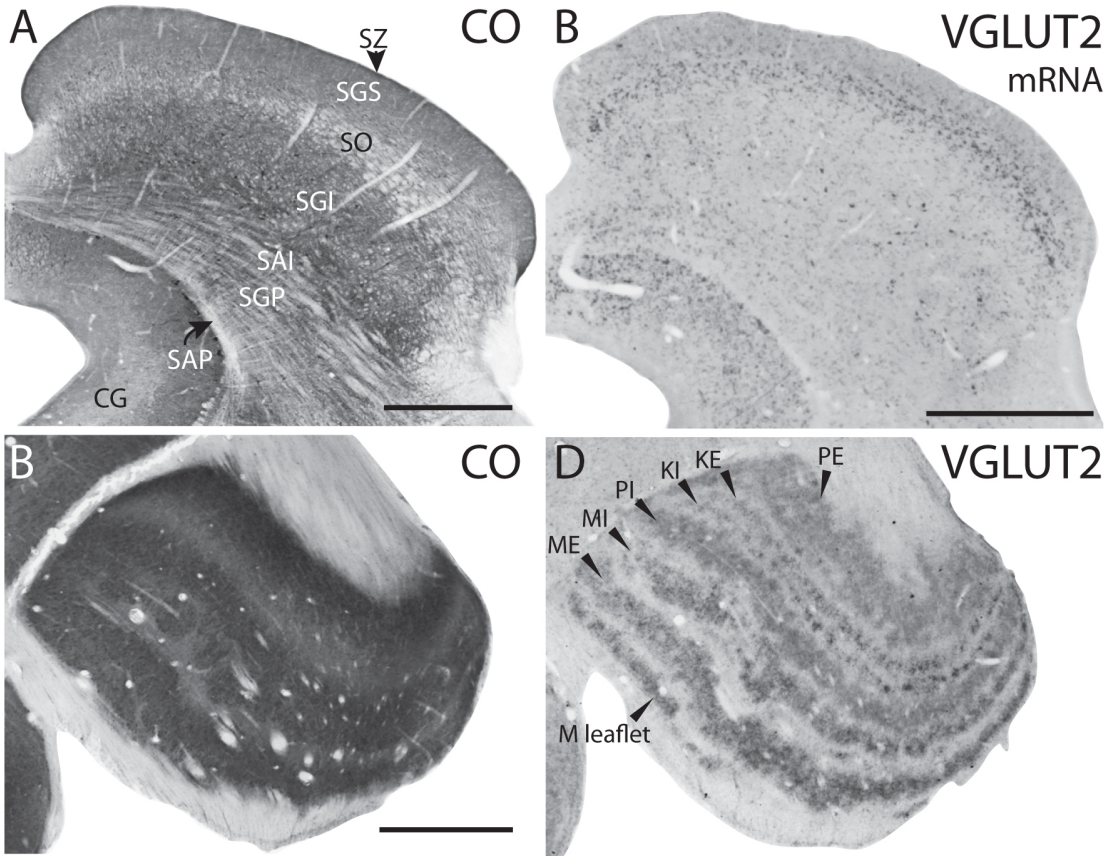


*Figure 3.2. Architecture of the superior colliculus and dorsal lateral geniculate nucleus in galagos. **A**. The superior colliculus can be subdivided into seven main layers based on cytochrome oxidase (CO): stratum zonale (SZ), stratum griseum superficiale (SGS), stratum opticum (SO), stratum griseum intermediate (SGI), stratum album intermediate (SAI), stratum griseum profundum (SGP), and stratum album profundum (SAP). **B**. Shows a cross section of the superior colliculus processed for VGLUT2 mRNA expression. Strong expression of VGLUT2 mRNA can be seen within the lower SGS. **C** and **D** show coronal sections of the lateral geniculate nucleus stained for CO (B), and VGLUT2 protein (D). The lateral geniculate nucleus can be divided into seven main layers that are apparent in VGLUT2 stained sections: external and internal parvocellular layers (PE and PI), external and internal konicellular layers (KE and KI), external and internal magnocellular layers, and an external magnocellular leaflet (ME leaflet).*

functions (See Kaas and Huerta, 1988; May 2006 for review). The SGS and the stratum griseum intermediale (SGI) can be distinguished by their dark CO staining relative to the

SO, and stratum album intermediate (SAI) (Fig. 3.2A). In galagos, the cells that project to the pulvinar complex are found within the lower SGS (Raczkowski and Diamond, 1981). These cells in the lower SGS also show strong expression of VGLUT2 mRNA (Fig. 3.2B) (Balaram et al., 2011), which correlates to VGLUT2 terminal labeling seen in the pulvinar complex. All injection sites included the SGS in the present study. A previously determined retinotopic map of the superior colliculus (Lane et al., 1973) was used to estimate the retinotopic locations of our injection sites.

3.4.1.2. The dorsal lateral geniculate nucleus

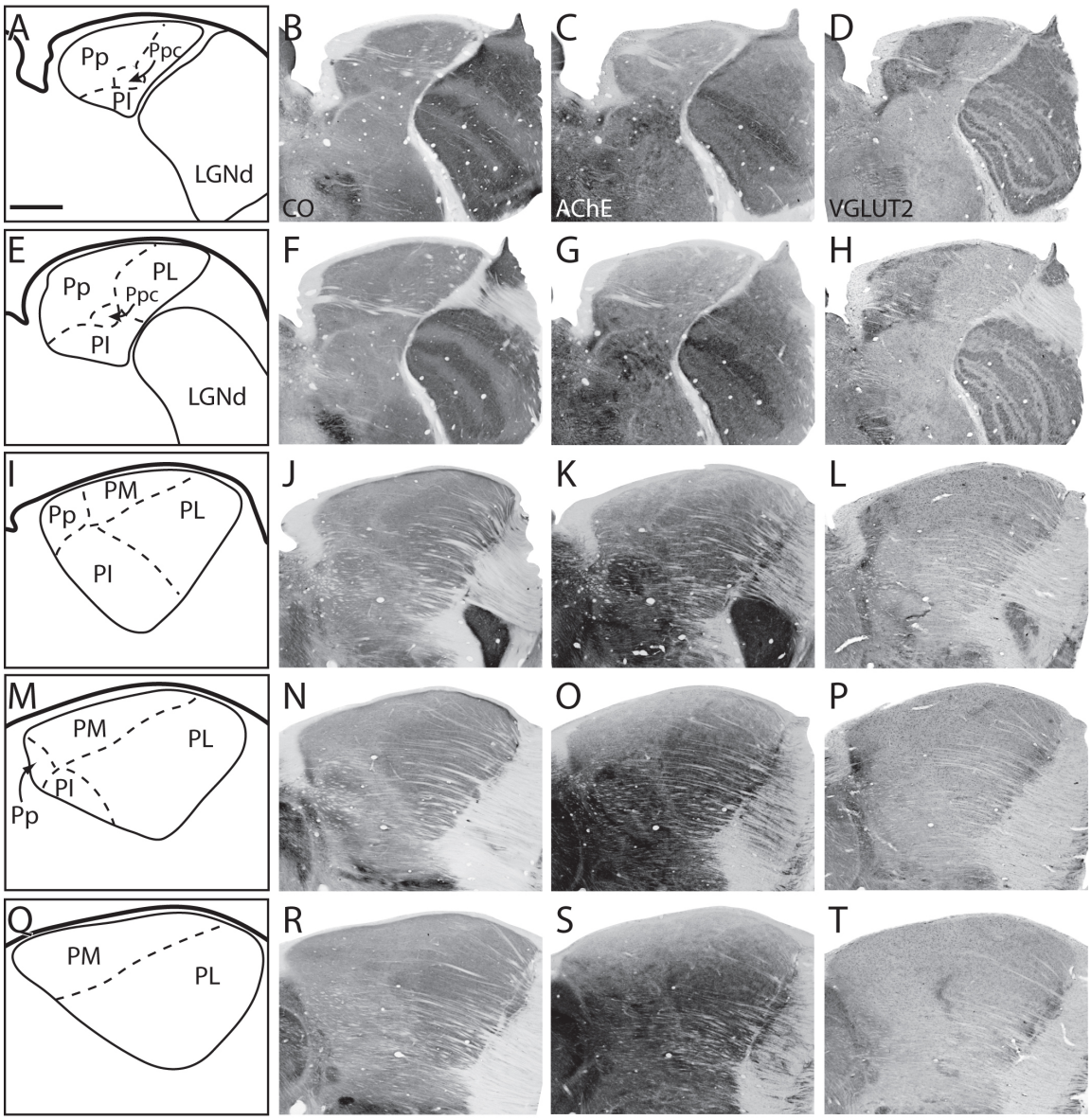
In galagos, the dorsal lateral geniculate nucleus (LGNd) lies ventrolateral to the pulvinar complex (Figs. 3.2A-B, 3.3A-H). In coronal sections, 6 main layers could be identified, including one magnocellular leaflet layer located most ventrally, two magnocellular (M) layers, and two dorsal parvocellular (P) layers separated by two koniocellular (K) layers. The K layers were easily identified as they stained darkly for VGLUT2 (Fig. 3.2C), but stained lightly for AChE or CO (Figs. 3.2B, 3.3F-G).

3.4.1.3. Pulvinar complex

In galagos, the pulvinar complex, which is part of the dorsal thalamus, extends as far caudal as the medial geniculate nucleus and as far rostral as the ventral posterior nucleus. The pulvinar of primates is typically divided into four main subdivisions, the anterior pulvinar, the medial pulvinar, the lateral pulvinar, and the inferior pulvinar (Stepniewska and Kaas, 1997; Stepniewska et al., 1999; Kaas and Lyon, 2007; Jones et al., 2007; Wong et al., 2009). Identifying nuclei or subdivisions within the pulvinar complex of galagos has been difficult (Wong et al., 2009), mainly because the typical staining techniques that reveal such nuclei in anthropoid primates have not been as

informative in galagos. In the present report, we defined borders between and within the medial, lateral, and inferior pulvinar, focusing on the caudal half of the pulvinar complex. While defining architectonic borders, and therefore nuclei within the pulvinar complex of galagos, remains difficult, the addition of sections processed for VGLUT2 proved to be useful.

VGLUT2 protein staining resulted in a robust border visible within the caudomedial aspect of the pulvinar complex (Fig. 3.3D,H and L). Based on comparative studies of VGLUT2 staining and superior colliculus projections, the region that stains darkly for VGLUT2 is part of the inferior pulvinar of anthropoid primates (See discussion). In the present report, we refer to this region as the posterior pulvinar because of its general location within the pulvinar complex, and also because this domain extends dorsally to the most dorsal aspect of the pulvinar complex—a region often previously attributed to the medial, or superior pulvinar in galagos (Glendenning et al., 1975; Symonds and Kaas, 1979; Diamond et al, 1992; Wong et al., 2009). The VGLUT2 staining region appears to be composed of two subdivisions (Fig. 3.4), and therefore we refer to these two regions as the posterior pulvinar (Pp) and the posterior central pulvinar (Ppc). At the most caudal extent, the posterior pulvinar, inferior and possibly parts of the lateral pulvinar are present (Fig. 3.3 A-D). In VGLUT2 stained sections, the medial half of the pulvinar stained darkly for VGLUT2 protein with two patches of VGLUT2 terminals that fused together ventrally (Figs. 3.3D, H, and 3.4). Caudally, the lateral aspect of the pulvinar stained weakly for VGLUT2 (Fig. 3.3D). This pattern of VGLUT2 staining was sometimes matched in cytochrome oxidase stained sections with dark CO staining corresponding to the dark VGLUT2 staining (Fig. 3.3B). However, the pattern


A
Pp
Ppc
Pl
LGNd
B
CO
C
AChE
D
VGLUT2
E
PL
Pp
Ppc
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T

*Figure. 3.3 Subdivisions of the pulvinar complex in prosimian galagos revealed in coronal sections of the pulvinar processed for CO, AChE, or VGLUT2. The line drawings on the far left column (**A, E, I, M, Q**) depict the borders within the pulvinar complex in coronal sections based on different staining procedures. Cytochrome oxidase (**B, F, J, N, R**) staining patterns reveal the medial pulvinar complex (**J, N, R**) because of its lighter CO staining intensity relative to surrounding subdivisions of the pulvinar complex. In ideal staining, the posterior region of the pulvinar can be demarcated from surrounding subdivisions by its darker CO staining pattern (**B** and **F**). AChE stained sections help indicate the medial pulvinar from surrounding subdivisions by the lighter AChE staining in this subdivision (**K, O, S**); however, there is little difference in staining patterns between the posterior, inferior, and lateral subdivisions. Dense VGLUT2 staining is present within the posterior pulvinar complex (**D, H, L, P**) located medially within the whole pulvinar complex. This staining pattern matches the pattern observed in CO under ideal CO staining procedures. Additionally, a protrusion in the most ventrolateral aspect of the posterior division is evident (**D, H**). Additionally, a region at the most lateral aspect of the lateral pulvinar stains darkly for VGLUT2 (**P** and **T**). This staining pattern is also evident within AChE stained sections (**K, O, S**) by slightly darker AChE staining, as well as an apparent septa between this region and the rest of the lateral pulvinar (S). Caudal sections are presented at the top of the panel, while rostral sections are presented progressively towards the bottom of the panel. Scale bar is 1mm.*

observed using CO and VGLUT2 was not noticeable in AChE stained sections. Progressing rostrally, the VGLUT2 darkly staining region is replaced by the medial pulvinar, which stains lightly for VGLUT2. As the medial pulvinar emerges, the posterior pulvinar exits medially (Fig. 3D, H, L, P, T). Again, this progression was also somewhat visible in ideally stained CO stained sections, but not apparent in AChE stained sections.

The lateral pulvinar lies along the lateral aspect of the pulvinar complex. PL can be distinguished from the medial pulvinar by its darker staining in CO and AChE stained sections (Fig. 3J, K, N, O, R and S). Determining the border between PL and PI was more difficult, but in ideally stained sections there was a slight contrast change in CO and AChE stained sections at the border of PL and PI (Fig. 3.3N). The medial pulvinar stains weakly for CO, and AChE (Fig. 3.3J, K, N, O, R, S) and was located mediodorsally within the rostral half of the pulvinar complex.

On the most lateral aspect of the pulvinar complex there is a thin strip of tissue that is separable from PL by what could be a thin septum (see Fig. 3.3R, S, O). This strip of tissue also stains more darkly for AChE (Fig. 3.3K, O, S) and VGLUT2 (Fig. 3.3P, T) and is reminiscent of the S subdivision of the pulvinar described by Gutierrez et al. (1995) in macaques, but few reports have described such a region in New World monkeys. In more rostral sections, (not shown), this strip remains thin, never widening. We did not observe tectal projections to this region.

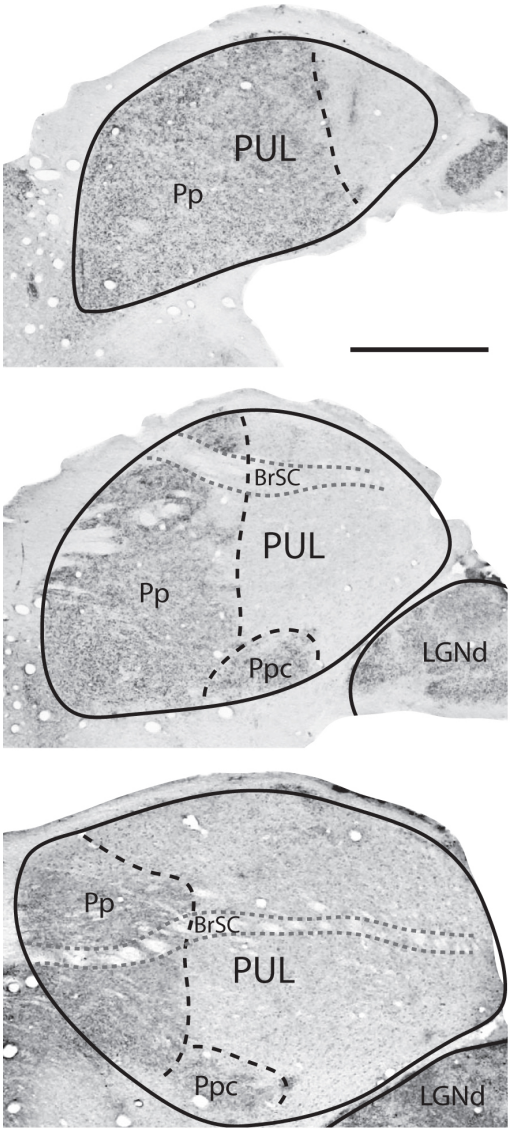


Figure 3.4. VGLUT2 staining in the caudal half of the pulvinar complex in galagos. Photomicrographs of VGLUT2 stained sections from the most caudal (top) to more rostral sections (bottom) through the pulvinar complex in caudal (top panel). Within the VGLUT2 staining region, to divisions are present. One large division along the medial aspect of the caudal pulvinar, the posterior pulvinar (Pp), and an additional, smaller division located ventrolaterally, the posterior central pulvinar (Ppc—tentative name). Scale bar is 1mm.

3.4.1.4. Other subcortical visual structures with superior colliculus connections

The parabigeminal nucleus is located along the lateral aspect of the brain stem just ventral to the caudal aspect of the superior colliculus and the brachium of the inferior

colliculus (Fig. 3.5E). This nucleus stains darkly in CO, AChE, and VGLUT2 preparations (Fig. 3.5E).

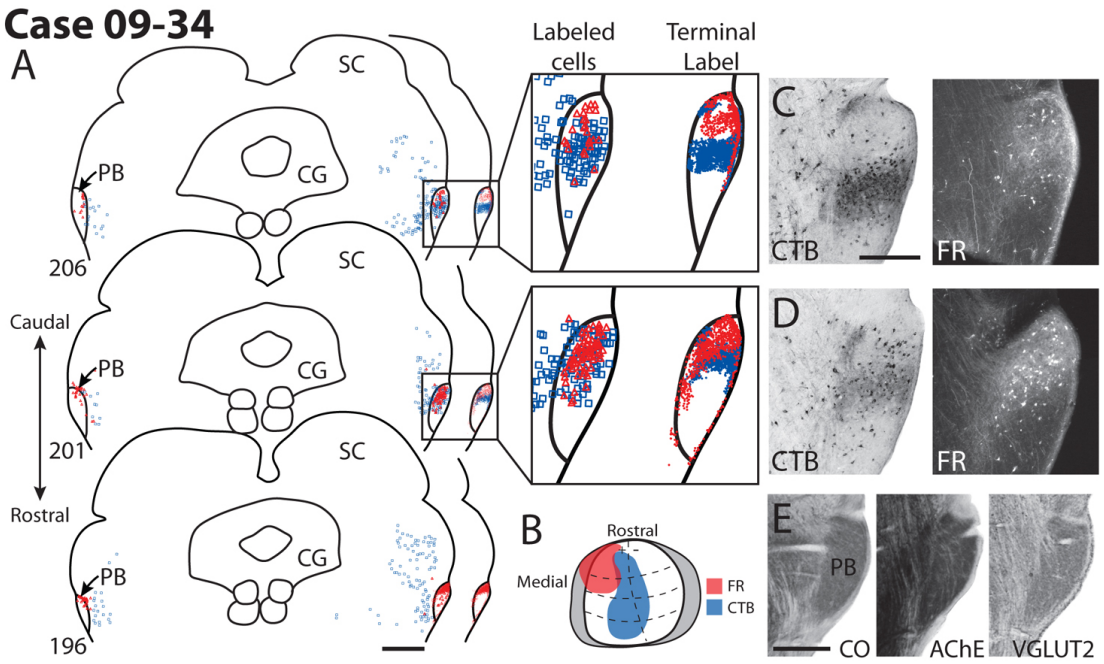


Figure. 3.5. Histological staining and terminal label after superior colliculus injections within the parabigeminal nucleus of galagos for case 09-34. ***A****. Reconstruction of the terminal label and retrogradely labeled cells within coronal brainstem sections containing the parabigeminal nucleus. The distribution of retrogradely labeled cells is presented to the left, while the terminal label is indicated in shadow reconstructions offset to the right. Enlargements of label are presented to the far left boxes of A. Blue squares represent retrogradely labeled cells while red triangles represent fluororuby labeled cells. CTB and FR terminal label are indicated by blue and red dots respectively.* ***B*** *Is a dorsal view reconstruction of the injection sites within the superior colliculus.* ***C****. Photomicrographs of the resultant CTB and FR label associated with section 206.* ***D****. Photomicrographs of the resultant CTB and FR label associated with section 201.* ***E****. Histological sections stained for cytochrome oxidase (CO), acetylcholinesterase (AChE), and vesicle glutamate transporter II (VGLUT2) of the parabigeminal nucleus (PB). Scale bare for A is 1mm, D and E is 0.25mm, and E is 0.5mm.*

The ventral lateral geniculate nucleus (LGNv) is located ventral and rostral to the lateral geniculate nucleus, and lateral to the ventral posterior nucleus. This nucleus stains heterogeneously for VGLUT2 and AChE, and darkly for CO. The nucleus limitans (Lim) is medial to the pulvinar complex and stains darkly for VGLUT2, and CO. The pretectal

nuclei that lie medial and caudal to the limitans and ventral to the rostral parts of the superior colliculus did not stand out in the present material. The substantia nigra is located medioventrally within the brainstem and projects to the superior colliculus in galagos (Huerta et al., 1991). Though these connections were identified, they are not described.

3.4.2. Connections of the superior colliculus

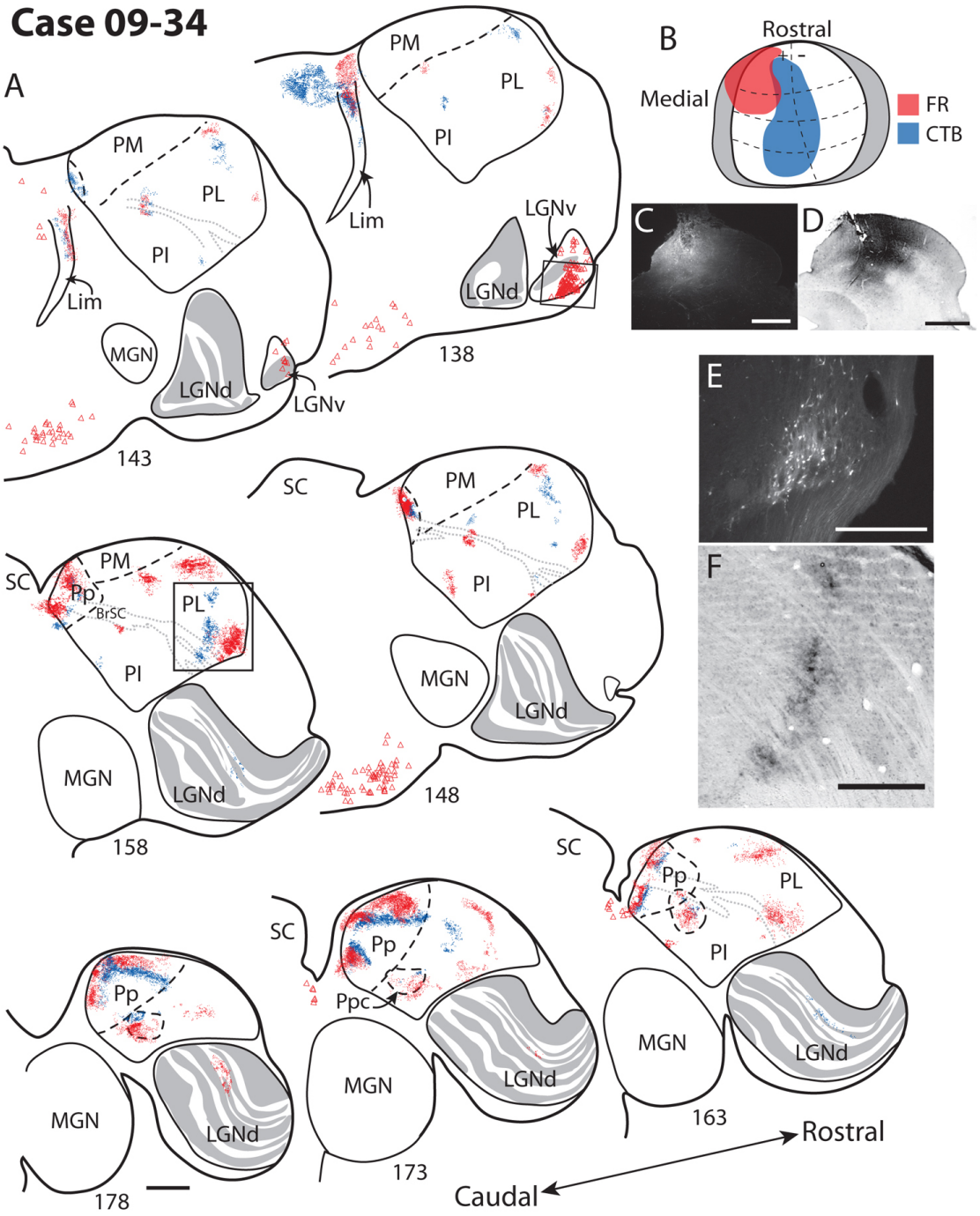
3.4.2.1. Projections to the pulvinar complex

Projections to the pulvinar complex were studied in 8 cases after placing injections of anatomical tracers of variable sizes and locations in the superior colliculus. We identified at least four, likely topographic, projections to the pulvinar complex after superior colliculus injections. Two of these projections were to two darkly staining VGLUT2 subdivisions of the pulvinar complex located within the caudomedial aspect, while two or three were to regions of the rostrolateral pulvinar that stained weakly for VGLUT2 and are likely within inferior and lateral subdivisions. All terminal projections were to the ipsilateral pulvinar complex, and no terminals were observed within the contralateral pulvinar after superior colliculus injections.

3.4.2.1.1. Projections to VGLUT2 staining divisions of the pulvinar complex

The results from cases 09-34 (Fig. 3.6) and 10-51 (Fig. 3.7) are especially informative as they had both CTB and FR injections in the superior colliculus, as well as sections processed for the VGLUT2 protein. In both cases 09-34 (Fig. 3.6), and 10-51 (Fig. 3.7), two clear patches of label were present within the two VGLUT2 staining domains in the caudal aspect of the pulvinar complex (Fig. 3.8). One larger, dense patch was located in the division we defined as the posterior pulvinar (Pp), and a second

*Figure 3.6. Reconstruction of the terminal label within the pulvinar complex after CTB and FR injections in the superior colliculus for case 09-34. A dorsal view reconstruction depicting the location of the injection sites within the superior colliculus (**A**) with red representing the fluoro ruby injection site, and blue representing the CTB injection site. Lines depicting the topographic layout of the superior colliculus were superimposed onto the reconstruction from results of Lane et al. The grey regions around the main SC circle represent the medial and lateral walls of the superior colliculus flattened and unfolded to the sides. **B.** Photomicrographs of the CTB and FR injection sites in coronal sections of the superior colliculus. **C.** Reconstruction of the terminal label within the brainstem and thalamus. Small red dots represent FR terminal label, while red triangles represent retrogradely labeled cells. Blue dots represent CTB terminal label, and blue squares represent retrogradely labeled cells. Solid lines indicate the borders of nuclei within the brainstem and thalamus, while dashed lines within the pulvinar complex represent the proposed borders determined using VGLUT2, CO, and AChE staining patterns. Pp is the posterior pulvinar, PI is the inferior pulvinar, PM is the medial pulvinar, LGNd is the dorsal lateral geniculate nucleus, LGNv is the ventral lateral geniculate nucleus, MGN is the medial geniculate nucleus, Lim is the limitans, Rt is the reticular nucleus. **D**. Photomicrographs of the terminal label depicted in the dashed boxes of C. Scale bars for A, C, and D are 1mm, E and F are 0.5mm.*



smaller patch was within what we identify as the posterior central pulvinar (Ppc). Within Pp, multiple patches of label were present (Fig. 3.8B and 3.8A, section 173). However, it is unclear if these multiple patches are a result of fiber tracts running through this

division, breaking up a single focus of superior colliculus projections within Pp, or are present because Pp has further subdivisions. This patchy appearance within Pp was also apparent in case 07-105, which also had a more rostral injection site within the portion of the superior colliculus representing paracentral vision (Fig. 3.9), but not in cases with more caudal injections in the portion of the superior colliculus representing peripheral vision (Figs. 3.10-3.13).

The CTB injection sites for cases 09-34 (Fig. 3.6) and 10-51 (Fig. 3.7) were almost identical in location, and included portions of both upper and lower visual quadrants. The FR injection sites in these two cases were similar in that they were more rostrally located within the superior colliculus than the CTB injection sites and therefore were within more central vision representations of the superior colliculus. The FR label within Ppc for both cases was located ventral to the CTB label, suggesting that central vision is represented ventrally within Ppc. Additionally, both the CTB and FR zones of label progressed laterally from more caudal to rostral locations within Ppc suggesting bands of isoeccentricity that run through Ppc in a rostrolateral trajectory. Finally, the pattern of label from the more rostral injections of FR was more pronounced in rostral Ppc than label from the more caudally placed injections of CTB. Thus, in case 09-34, the CTB label was quite dense caudally, but less dense rostrally (Fig. 3.6 sections 178-163), and in case 10-51, CTB terminations were in the most caudal section of Ppc (Fig. 3.7 section 133), but FR terminal label in Ppc did not emerge until the subsequent section (Fig. 3.7 section 138). This suggests that central vision is represented rostrally within Ppc and peripheral vision is represented caudally.

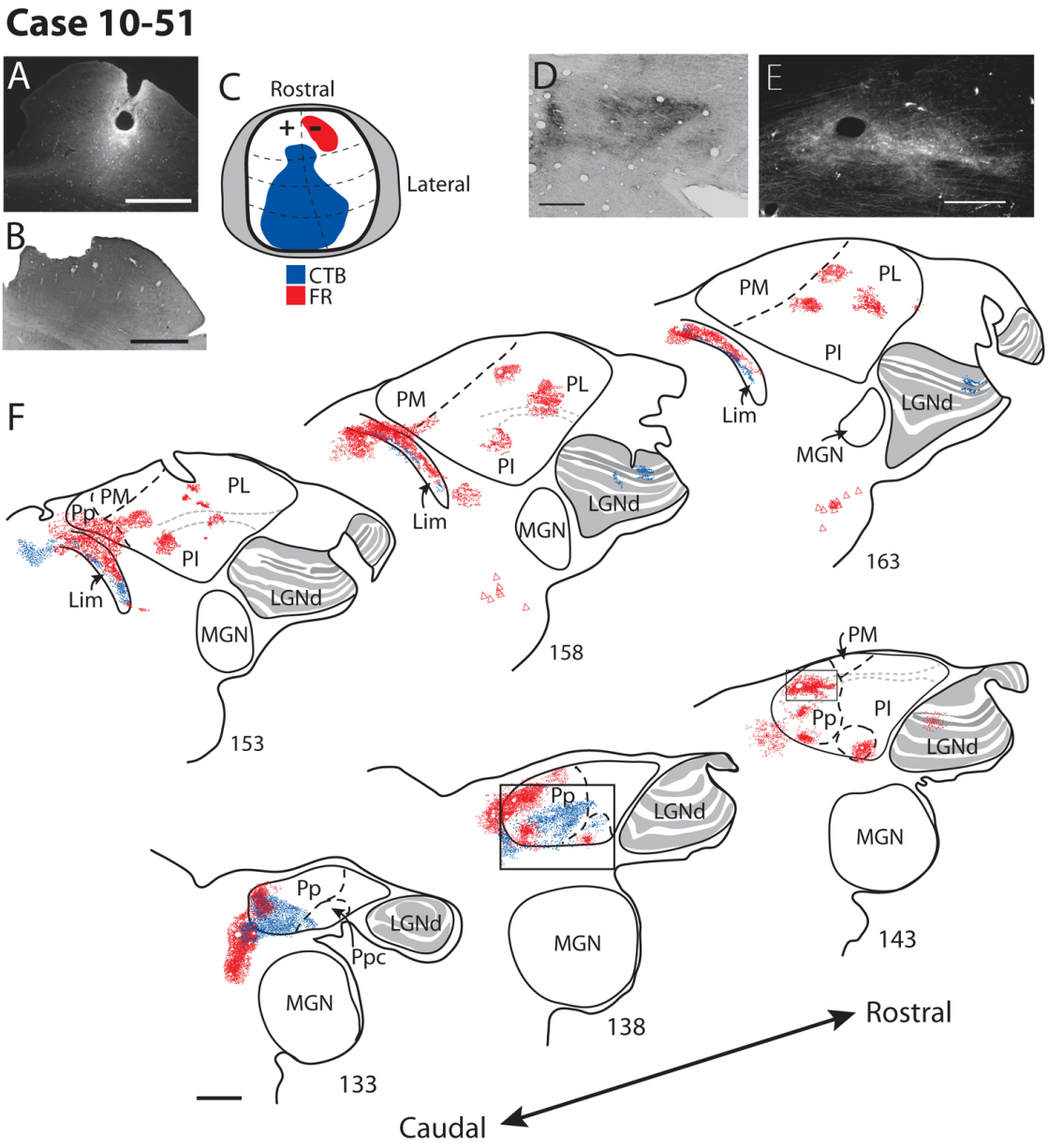


Figure 3.7. Reconstruction of terminal label within the pulvinar complex after a superior colliculus injection in case 10-51. ***A*** *and* ***B*** *are photomicrographs of the fluoro ruby (FR), and cholera toxin subunit B (CTB) injection sites in coronal sections within the superior colliculus.* ***C*** *shows the dorsal view reconstruction of the FR and CTB injection sites throughout the flattened superior colliculus.* ***D*** *and* ***E*** *are close up photomicrographs of CTB and FR terminal label in sections 138 and 143 shown in* ***F*** *respectively.* ***F*** *is the reconstruction of terminal CTB (blue dots) and FR (red dots) terminal label within the pulvinar complex. Retrogradely labeled FR cells are also shown (red triangles). Scale bars for A, B and F is 1mm, D is 0.5mm and E is 0.25mm.*

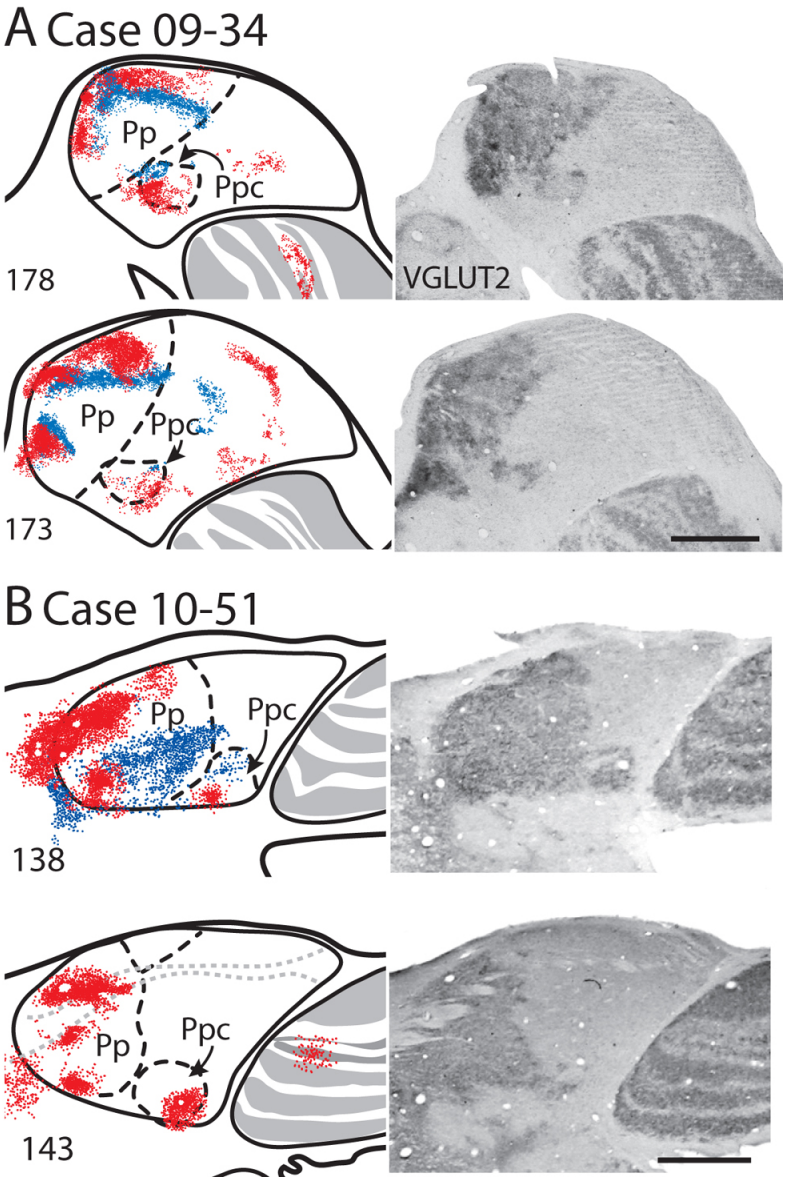


Figure 3.8. Close up view of the terminal label within sections 173 and 178 from case 09-34 and sections 138 and 142 of case 10-51 with adjacent VGLUT2 stained sections. Two patches of label for each tracer are noticeable. One set of isolated patches of CTB and FR terminal label are within the larger medial body of the VGLUT2 staining region, which we have tentatively named posterior pulvinar (Pp). Additional patches of terminal label are present within the protrusion of VGLUT2 staining off the most ventrolateral aspect of Pp, for which we tentatively name posterior central pulvinar (Ppc). Scale bar is 1mm.

The retinotopic organization within the larger Pp was less clear. Yet, the patches of terminal label within Pp for different injection sites in the superior colliculus did not

overlap much, suggesting that a topographic organization is present. The terminal label, for most cases, was present throughout the full rostrocaudal extent of Pp, therefore, making it difficult to determine the presence of a rostral/caudal topographic pattern (Figs. 3.8-3.13). Possibly, the caudal superior colliculus projects to ventromedial Pp, while the rostral aspect projects more to dorsolateral Pp.

3.4.2.1.2. Projections to non- or weakly-VGLUT2 staining divisions of the pulvinar complex

Determining borders between other subdivisions of the pulvinar complex was difficult. Therefore, we describe patches of labeled terminals outside of the VGLUT2 region based on their relative locations within the pulvinar. There were usually up to two patches of terminations in the most lateral portions of the pulvinar, one dorsal and one ventral (Figs. 3.6, 3.7, 3.9 and 3.10 but not Figs. 3.12 and 3.13), and one additional patch located more medially (Figs. 3.6-3.13). The more medial patch may be within the inferior pulvinar, and the more lateral patches are likely within the lateral pulvinar.

The most lateral tectal termination zone within the pulvinar is retinotopically organized. Injections within the representation of the upper visual quadrant in the superior colliculus labeled terminals lateral to those produced by injections within the lower visual quadrant representation (Fig. 3.6). This pattern was apparent when we compared the locations of terminal label with respect to the most lateral border of the pulvinar. For example, cases 07-105 (Fig 3.9) and 09-03 (Fig. 3.10), with injections at the extreme upper field representation produced labeled terminals along the lateral aspect of the lateral pulvinar, while cases 10-51 (Fig. 3.7) and 11-61 (Fig. 3.12) with injection sites located toward the lower visual field representation produced terminal label more medially.

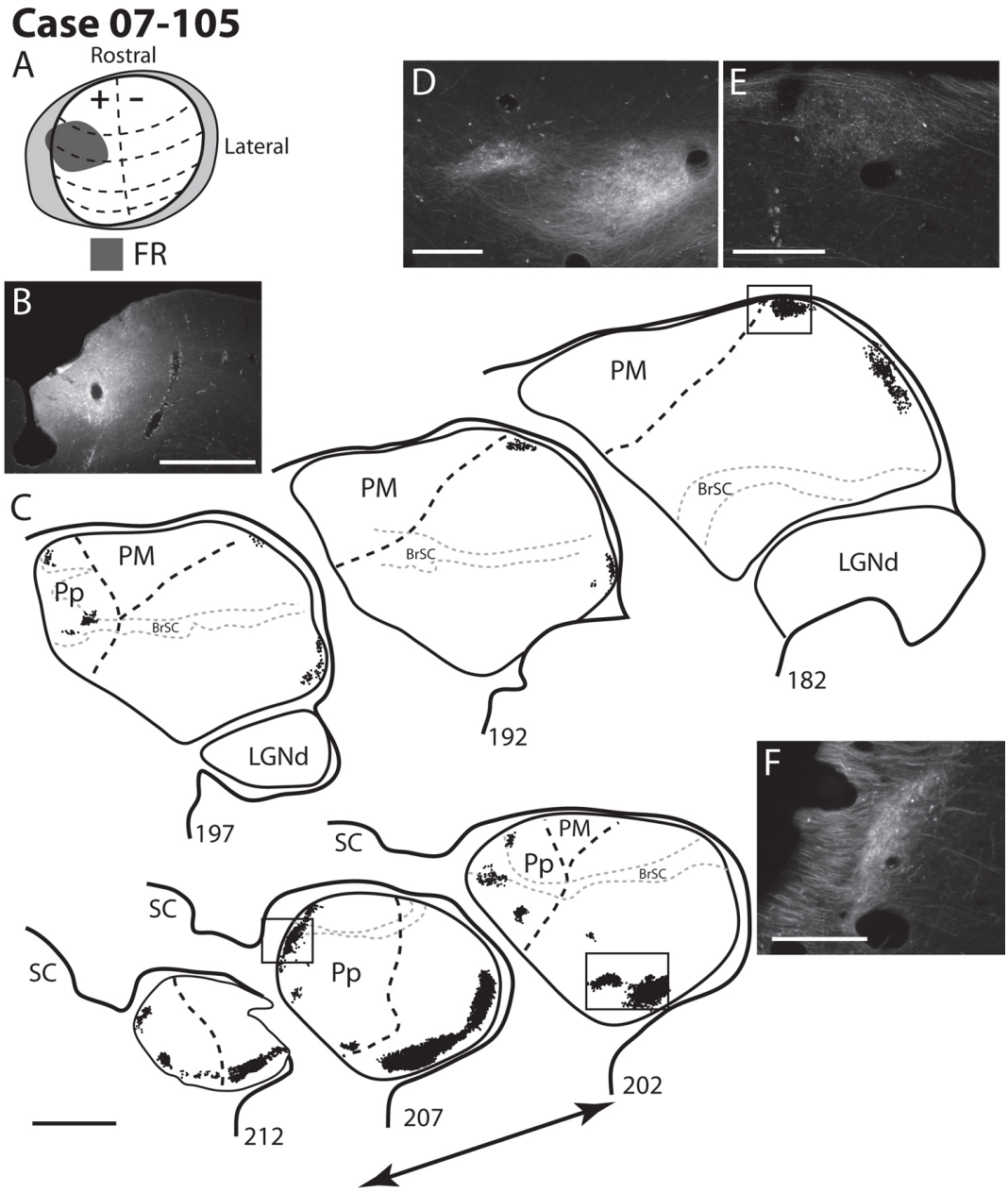


*Figure. 3.9. Terminal label within the pulvinar complex after a fluororuby injection into the medial superior colliculus of case 07-105. **A** show the dorsal view reconstruction of the extent of the injection site within the superior colliculus. **B**. is a photomicrograph of a coronal section through the superior colliculus at the level of the injection site. **C**. Reconstruction of the distribution of terminal label within the pulvinar complex. Black dots represent terminal label, dashed back lines represent borders within he pulvinar complex as defined by cytochrome oxidase. The grey dashed lines represent the location of the brachium of the superior colliculus (BrSC). **D**, **E**, and **F** are photomicrographs of the terminal label within sections 202, 182, and 202 respectively. Scale bar for B and C is 1mm. Scale bar for D, E and F is 0.25mm.*

Two patches of labeled axons within the lateral terminal zone of the pulvinar were not present for all cases. For instance in case 09-34, the FR label does form two patches, one ventrally and one dorsally (See Fig. 3.6 sections 163, 158, 148 for example); however the CTB terminal label formed only one continuous band of label (Fig. 3.6 sections 158, 148). The continuous band could reflect the large injection site, which covered almost the full rostral to caudal extent of the superior colliculus. Two patches, one ventral and one dorsal, were also present in cases 10-51 (Fig. 3.7), 07-105 (Fig. 3.9), and 11-27 (Fig. 3.11), but two terminal patches were less apparent in cases 09-03 (Fig. 3.10), 11-61 (Fig. 3.12) or 11-41 (Fig. 3.13). The difference between these cases is that the injection sites for cases with two clear patches were located rostrally within the superior colliculus, while the other cases had injection sites located caudally within the superior colliculus. This difference suggests that peripheral visual field representations adjoin within the more caudal location of the pulvinar. Finally, in case 10-51 (Fig. 3.7), CTB label was only present within the caudal pulvinar divisions that stain darkly for VGLUT2. No CTB terminal label was noticeable in the more rostral and lateral zones. As the superior colliculus injection in this case was the most superficial compared to all other cases where terminal label is observed, the results suggest that projections to the rostral lateral pulvinar may originate from deeper sublayers in the superior colliculus than those that project to the posterior pulvinar. The terminal patch within the medial aspect of the pulvinar (outside of the posterior pulvinar subdivisions) was often much smaller than patches observed more laterally (Figs. 3.6-3.13).

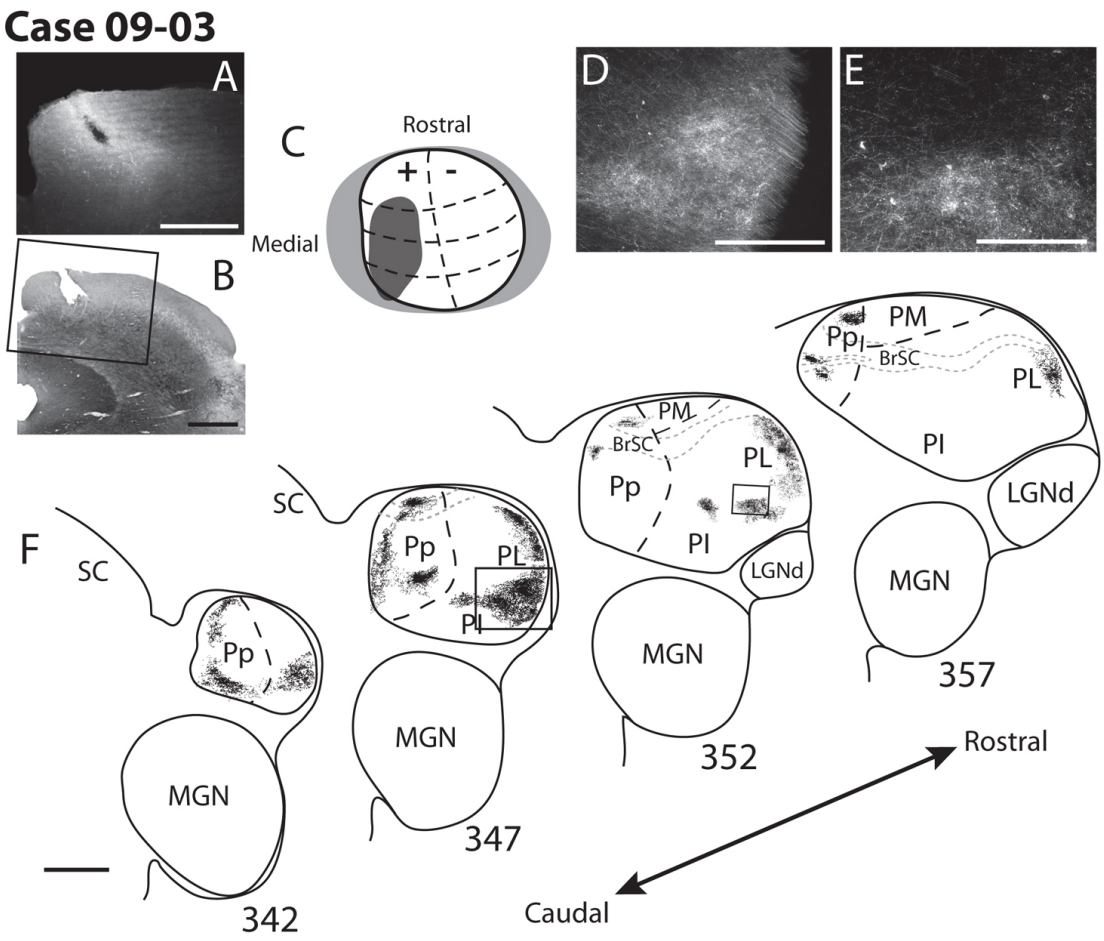


*Figure. 3.10. Reconstruction of terminal label within the pulvinar complex after an injection of FR into the superior colliculus for Case 09-03. **A** shows the FR injection site in a coronal section of the superior colliculus. **B** is a cytochrome oxidase section adjacent to the section in A. The boxed in area depicts the area of overlap between A and B. **C** shows the location of the injection site on a dorsal view reconstruction of the superior colliculus. **D** and **E** are photomicrographs of the terminal label in sections 347 and 352 in F respectively. **F** is the reconstruction of terminal label within the pulvinar complex with borders determined using CO stained sections. Scale bars for A, B and F are 1mm, D is 0.5mm and E is 0.25mm.*

In all cases, no terminal label was observed within the medial pulvinar, as defined by CO and AChE staining. This observation is consistent with previous reports in galagos (Glendenning et al., 1975), as well as other primates (Stepniewska et al., 1999; Stepniewska et al., 2000).

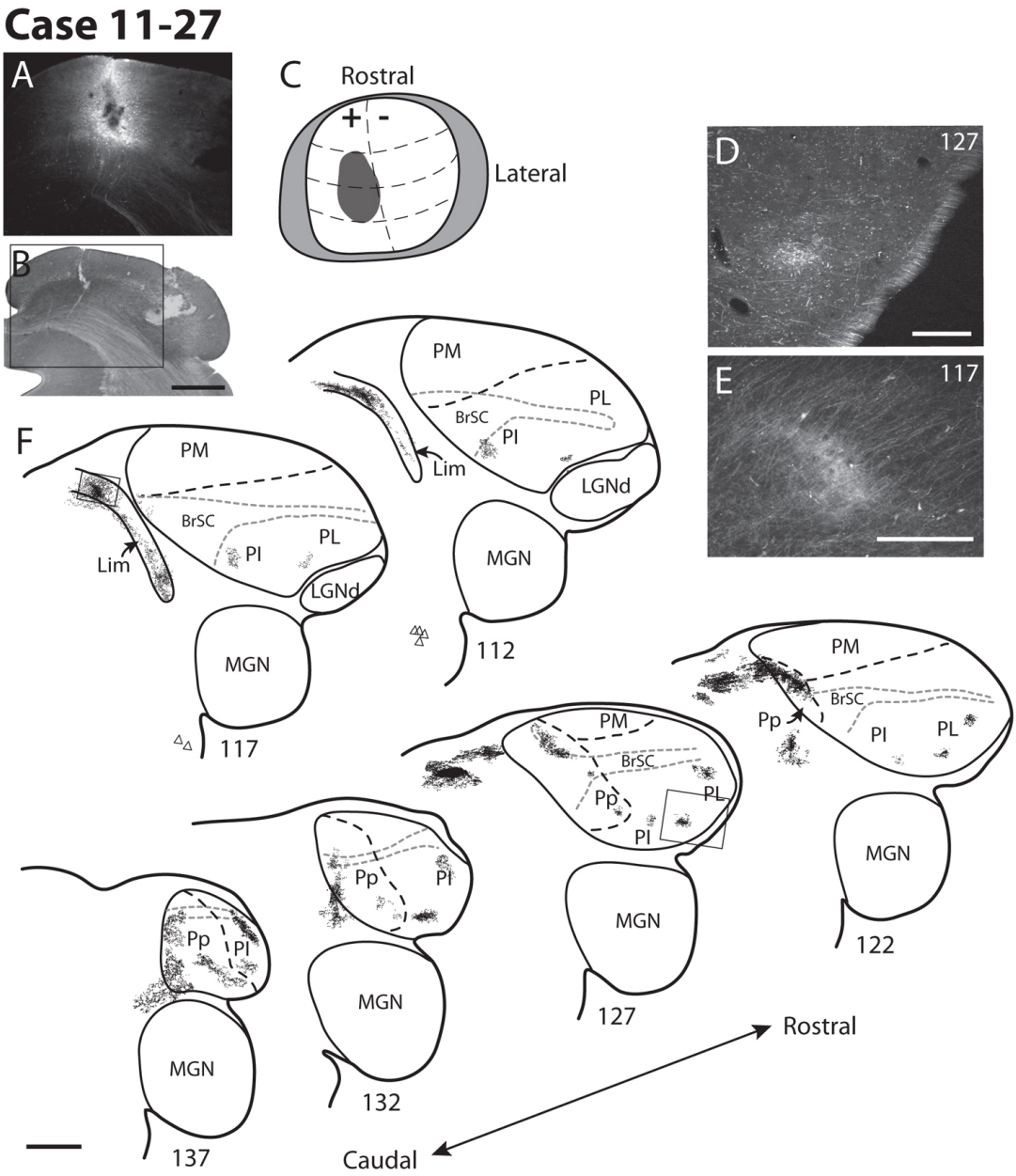


Figure 3.11. Location of terminal label within the pulvinar complex after a FR injection within the superior colliculus in case 11-27. ***A****. The FR injection site located within the superior colliculus in a coronal section.* ***B****. Shows the adjacent cytochrome oxidase section showing the location of the injection site location. The black box represents the extent of overlap between A and B.* ***C****. A dorsal view reconstruction of the flattened superior colliculus with the location of the injection site indicated by the grey oval.* ***D*** *and* ***F*** *are photomicrographs of the terminal label depicted in sections 127 and 117 of* ***F.*** ***F*** *shows the reconstructed sections of the brain stem and thalamus with the FR terminal label. Scale bar is 1mm for A, B and F, and 0.25mm for D and E.*

In summary, topographic projections from the superior colliculus terminate within two caudal divisions of the pulvinar that stain darkly for VGLUT2 protein. One patch of labeled terminals was located medially within a region we define as Pp (or PIp of other primates), and the second patch was located ventrolaterally within a region we define as Ppc (possibly PIcm of other primates). At least two additional terminal patches of label were located in more rostrolateral locations to those observed within the VGLUT2 stained regions. Projections to the most lateral zone were retinotopically organized.

3.4.2.2. Projections to the dorsal lateral geniculate nucleus

Projections to the dorsal lateral geniculate nucleus were to the K layers (Figs. 3.6 and 3.7), as well as to interlaminar zones between the external and internal magnocellular layers, and between the internal magnocellular and parvocellular layers (Fig. 3.11). These findings are consistent with previous results in galagos (Harting et al., 1986) and other primates (Harting et al., 1978; Stepniewska et al., 1999). We did not find additional terminal label within parvocellular or magnocellular layers of the dorsal lateral geniculate nucleus, indicating that pretectal nuclei and striate cortex were not contaminated by our superior colliculus injections (Symonds and Kaas, 1978; Harting et al., 1986). Additionally, injections in a more rostral location within the superior colliculus resulted in terminal label more caudally positioned in the dorsal lateral geniculate nucleus than caudal superior colliculus injections (Figs. 3.6 and 3.7). This observation is consistent with the retinotopy of the lateral geniculate nucleus of galagos, as central vision is represented caudally and peripheral vision represented rostrally (Symonds and Kaas, 1978).

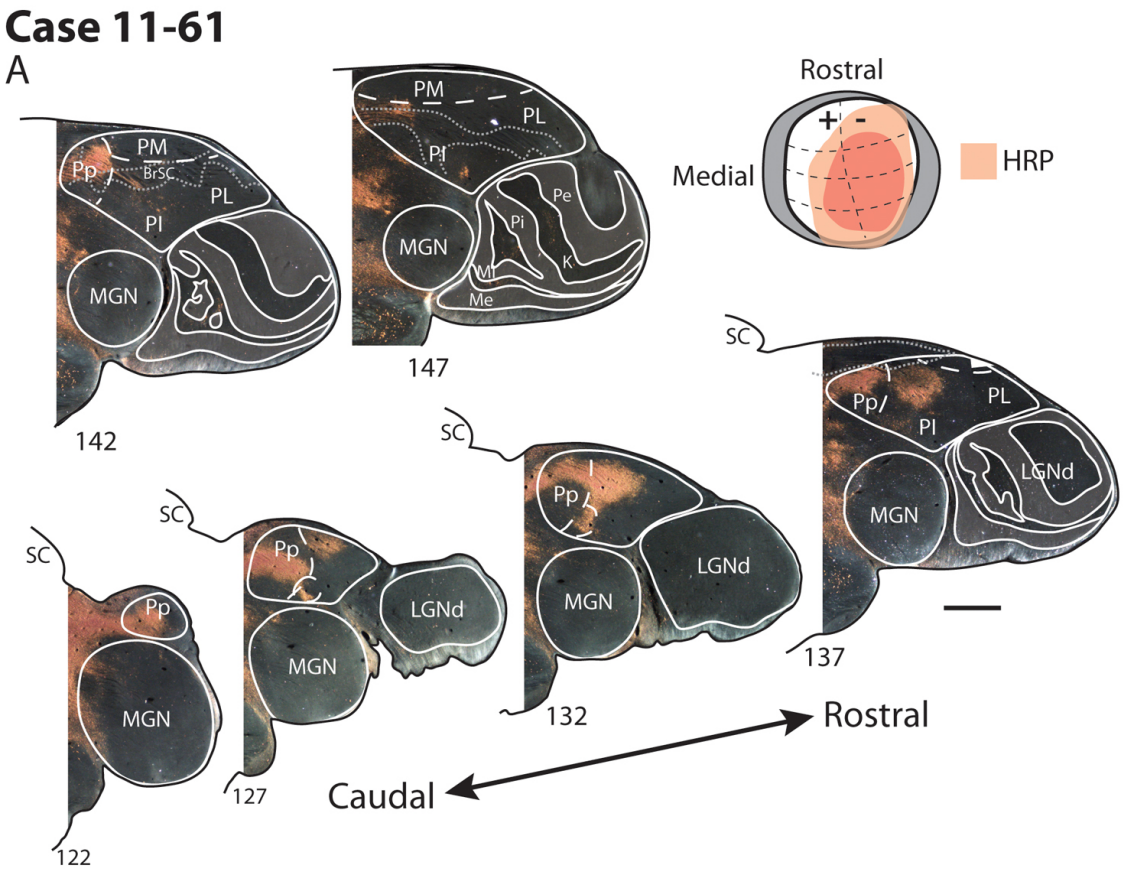


*Figure 3.12. Terminal label within the pulvinar complex after WGA-HRP and FR injections into the superior colliculus of galago case 11-61. The extent of the tracer spread is depicted in the dorsal view reconstruction of the superior colliculus **A**. **B** Dark field photomicrographs of WGA-HRP label within the pulvinar complex with borders determined using cytochrome oxidase staining shown in white. Scale bar for B is 1mm.*

3.4.2.3. Connections with the parabigeminal nuclei

Both retrogradely labeled cells as well as labeled terminals were located within the ipsilateral parabigeminal nucleus, while only retrogradely labeled cells were present within the contralateral parabigeminal nucleus for all cases (Fig. 3.5). This observation is consistent with those from other primates (Harting et al., 1980; Baizer and Whitney, 1991), and is consistent with the connections revealed by tracer injections placed in the parabigeminal nucleus of galagos (Diamond et al., 1992). Additionally, the patches of terminal label from two separate superior colliculus injection sites in the same cases were

distinct within the parabigeminal nucleus, with label from the more rostral injection located rostrally and dorsally, and label from the caudal injection located caudally and ventrally. This type of topography has been reported for cats and Old World monkeys (Sherk, 1979; Rodán et al., 1983; Baizer and Whitney, 1991). The location of retrogradely labeled cells within the parabigeminal nucleus was less organized, with retrogradely labeled cells occupying a broader area than the area occupied by the terminal label, yet the general topographical pattern was similar to that observed for the terminal label, and the majority of cells were located in the same vicinity as the terminal label. Within the contralateral parabigeminal nucleus, far fewer cells were observed than those observed ipsilaterally.

3.4.2.1. Connections with other subcortical visual structures

Terminal label and retrogradely labeled cells were observed within the ventral lateral geniculate nucleus (Fig. 3.6), and terminal label was observed within the pretectal nuclei (Fig. 3.7), nucleus limitans (Figs. 3.6, 3.7, 3.11) and suprageniculate nucleus (Fig. 3.12), while retrogradely labeled cells were observed in the substantia nigra (Figs. 3.6, 3.7, 3.11, 3.12, 3.13).

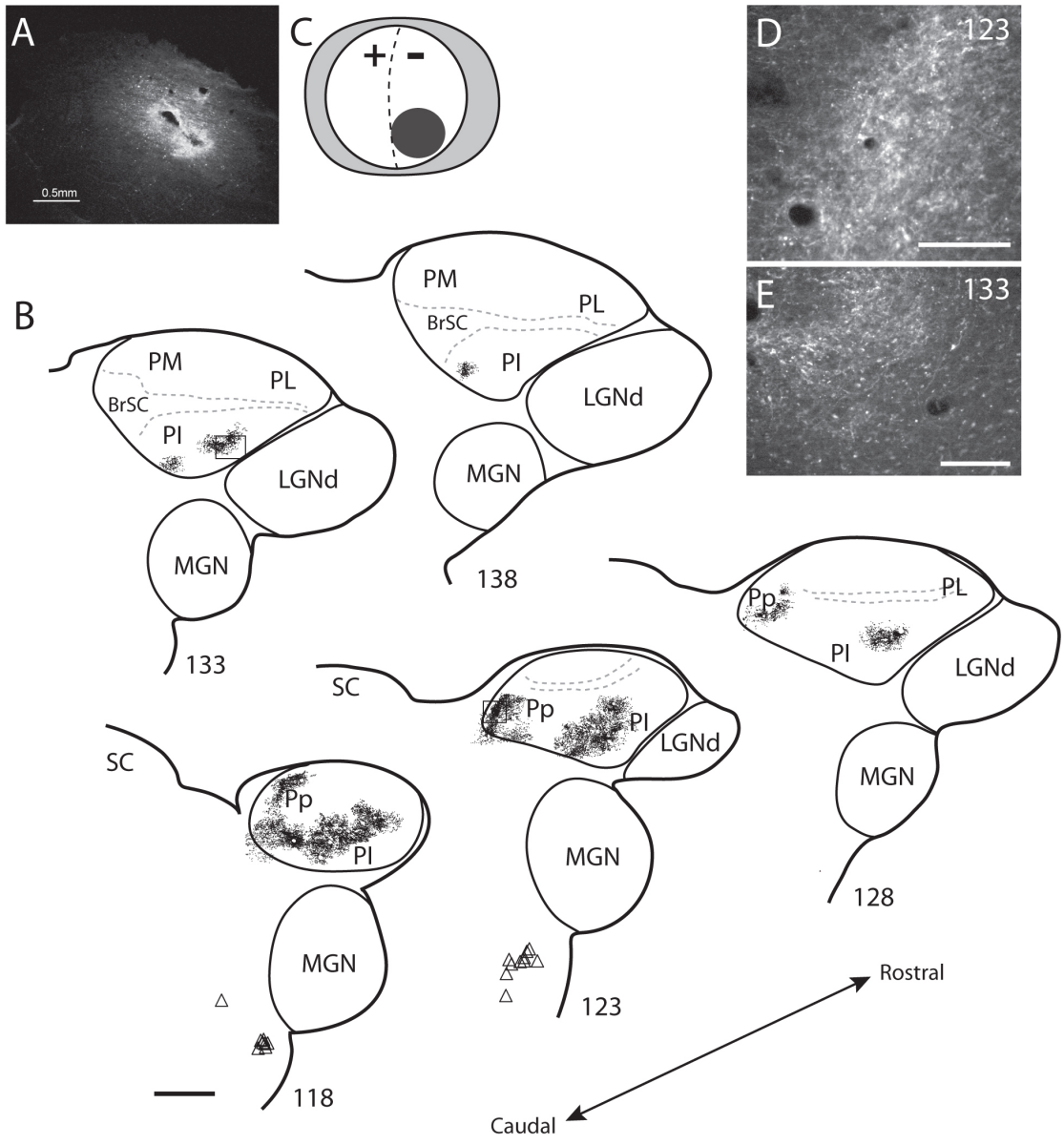


Figure 3.13. The distribution of terminal label within the pulvinar complex after an injection in the caudolateral aspect of the superior colliculus of a galago, case 11-41. ***A*** *is a photomicrograph of the FR injection site within the superior colliculus. The location is within the caudal and lateral aspect of the superior colliculus representing the peripheral upper field.* ***B*** *is a the reconstruction of terminal label within the pulvinar complex.* ***C*** *and* ***D*** *are photomicrographs of terminal label within sections 123 and 133 of B respectively. Scale bars for A and B are 1mm, Scale bars for A is 0.5mm, B is 1mm, C and D are 0.25mm.*

3.5. DISCUSSION

In the present study, we compared connection patterns of the superior colliculus with architectural characteristics of the pulvinar complex of prosimian galagos. We determined that two, topographically organized projections from the superior colliculus terminate in two subdivisions of the pulvinar that express large amounts of the vesicular glutamate transporter VGLUT2. One such projection is to a larger medial division, which we tentatively name the posterior pulvinar (Pp), and the second projection is to a smaller division located more ventrolaterally, which we tentatively name the central posterior pulvinar (Ppc). Alternatively, perhaps with more evidence, these nuclei could be named after their proposed homologues in the inferior pulvinar of anthropoid primates; PIp for Pp and PIcm for Ppc.

The association between superior colliculus projections and VGLUT2 staining suggests that the use of VGLUT2 for synaptic transmission defines two pulvinar locations for superior colliculus terminations in galagos that can be similarly identified in other mammals. This possibility is supported by the expression of VGLUT2 mRNA in cells of the lower SGS in the colliculus (Balaram et al, 2011 and see Fig. 3.2), which are known to project to the inferior pulvinar in primates (Raczkowski and Diamond, 1981). Additionally, at least two likely topographically distributed projections terminate within more rostrolateral aspects of the pulvinar complex. These projections are to regions of the pulvinar that stain weakly for VGLUT2 protein, and are likely to be divisions within the inferior and (or) lateral pulvinar. Thus, projections from the superior colliculus to the pulvinar complex that do not depend on VGLUT2 for synaptic transmission also exist in galagos.

Our injections also revealed superior colliculus connections with the dorsal and ventral lateral geniculate nuclei, pretectum, parabigeminal nuclei, limitans, suprageniculate nucleus, and the substantia nigra. Projections from the superior colliculus to the dorsal lateral geniculate nucleus were topographically organized, with more rostral superior colliculus injections resulting in terminal label located caudally within the dorsal lateral geniculate, while caudal injections resulted in terminal label more rostrally. Terminations were found both within K layers of the dorsal lateral geniculate nucleus, as well as to intralaminar zones between MI and ME, and MI and PI. These results are similar to those of previous reports (Harting et al., 1986). Bi-directional connections were also observed between the superior colliculus and the ipsilateral parabigeminal nucleus, as described previously (Diamond et al., 1992). The contralateral parabigeminal nucleus also sends projections to the superior colliculus, consistent with results described in squirrels (Holcombe and Hall, 1981; Baldwin et al., 2011), and anthropoid primates (Harting et al., 1980; Baizer and Whitney, 1991). We were able to determine that the projection pattern to and from the parabigeminal nucleus is topographically organized, with more rostral superior colliculus injections terminating in more rostral locations of the parabigeminal nucleus compared to caudal injections as reported for other species (Sherk, 1979; Rodán et al., 1983; Baizer and Whitney, 1991). Connections were also observed between the superior colliculus and the ventral lateral geniculate nucleus, the nucleus limitans, pretectum, and substantia nigra, as previously reported in galagos (Glendenning 1975; Huerta et al., 1991; Diamond, 1992).

3.5.1. Previous reports on galago pulvinar organization

Our present findings provide new insights on how the pulvinar complex is organized in mammals and how the complex pattern of pulvinar nuclei in anthropoid primates evolved. Histological staining procedures within the pulvinar complex of galagos have proven to be less robust and therefore less helpful in delineating subdivisions (Beck and Kaas, 1998; Wong et al., 2009) than they have been for anthropoid primates (Cusick et al., 1993; Gutierrez et al., 1995; Stepniewska and Kaas, 1997; Adams et al., 2000; Jones, 2007), making the assignment of homologues between prosimians and anthropoids difficult (Beck and Kaas, 1998; Wong et al., 2009).

Previous studies on the organization of the pulvinar complex in galagos used both anatomical markers, such as Nissl substance, cytochrome oxidase, and myelin, as well as differences in cortical and tectal connections to demarcate pulvinar subdivisions (Glendenning et al., 1975; Raczkowski and Diamond, 1980, 1981; Symonds and Kaas, 1978; Wall et al., 1982; Wong et al., 2009). Among the first studies, Glendenning et al, (1975) subdivided the pulvinar complex into inferior and superior divisions with the border between such divisions marked by the brachium of the superior colliculus. Glendenning observed that the caudal aspect of the pulvinar complex receives projections from the superior colliculus, mainly within their defined inferior pulvinar division, and few or no projections were found within the superior pulvinar division except after very large injections into the superior colliculus (see Fig. 11 section 84 of Glendenning et al., 1975 for example). Later Raczkowski and Diamond (1981) showed that injections into the superior pulvinar retrogradely labeled cells within the superficial layers of the superior colliculus (Figs. 11, 13, and 13 of Raczkowski and Diamond, 1981). Finally,

further evidence provided by Diamond et al., 1992, showed that the superior colliculus projects most densely to the caudal half the pulvinar complex, but also more rostrally, even into the classically defined superior pulvinar division above the brachium of the superior colliculus (See Fig. 5 of Diamond et al., 1992). In our current study, we also found terminal label above the brachium of the superior colliculus.

Other studies in galagos suggested that the superior pulvinar can be divided into a medial pulvinar (PM) and a lateral pulvinar (PL) based on differences in cortical connections with visual structures, such that PM does not share many connections with visual cortical areas while PL does (Symonds and Kaas, 1979; Wall et al., 1982; Wong and Kaas, 2008). We found that the superior colliculus projects only within the lateral half of the classically defined superior pulvinar, and this termination zone is likely PL. However, this terminal zone could also be within a subdivision of the inferior pulvinar that traverses the brachium of the superior colliculus. This lateral terminal zone appears to be topographically organized, similar to descriptions of the lateral pulvinar (superior pulvinar) of Symonds and Kaas (1978), with upper visual field represented laterally and the lower visual field represented medially. The terminal label zone from any given injection site in the superior colliculus reveals a band of isoecentricity that shifts slightly through the rostrocaudal extent of the pulvinar complex, similar to descriptions by Symonds and Kaas (1978) (See Fig. 3.9 sections 197 to 182 for example). In many reports (Symonds and Kaas, 1978; Wall et al., 1982), the peripheral visual field was suggested to be represented ventrally and dorsally, while the central visual field was suggested to be represented centrally within the lateral aspect of the pulvinar. While it

was difficult for us to confirm this type of topography in our present study, our results were largely

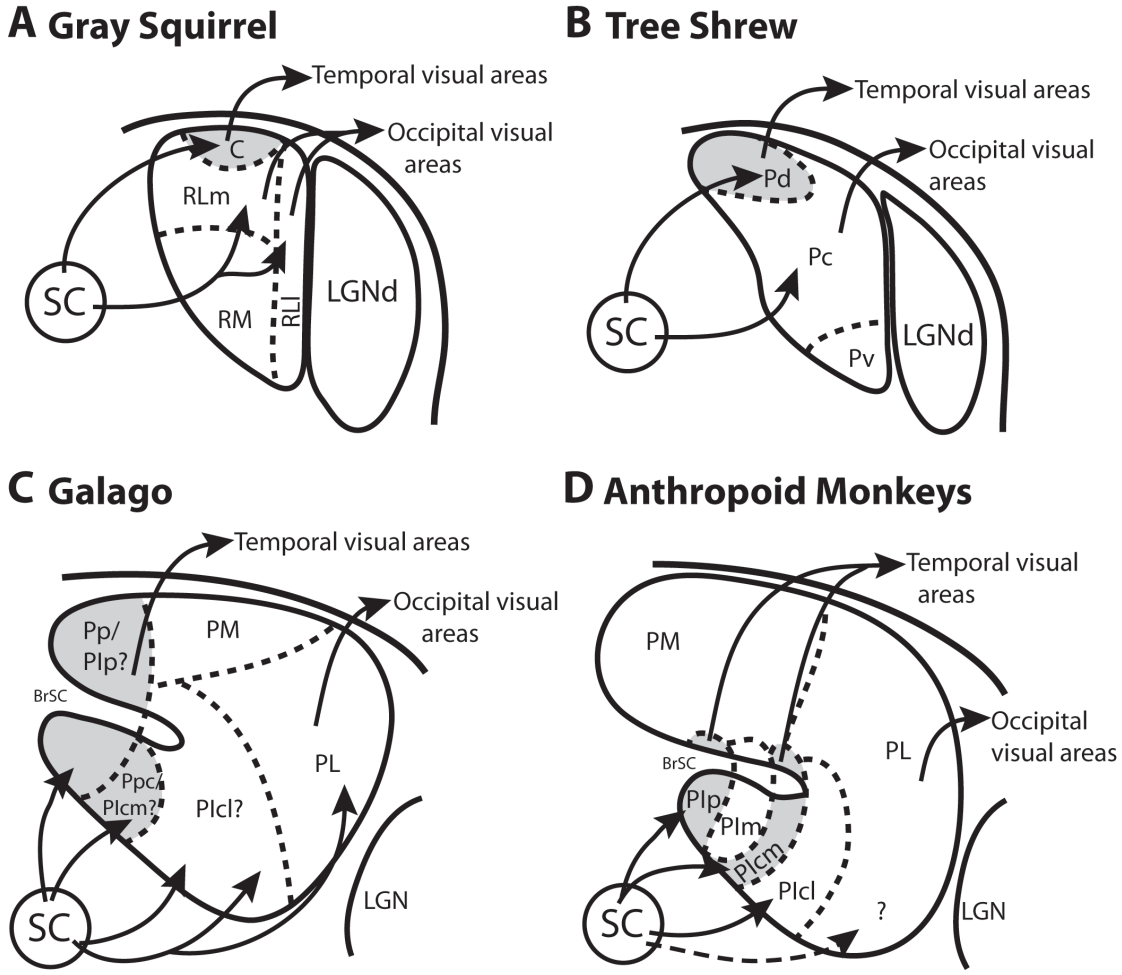


Figure 3.14. Organization schemes of the pulvinar complex with superior colliculus (SC) inputs for various members of the Euarchontoglires clade. Grey regions within the pulvinar complex stain darkly for VGLUT2. ***A****. Gray squirrels, based on descriptions from Baldwin et al., 2011. C is the caudal pulvinar, RLm is the rostral lateral medial pulvinar, RLl is the rostral lateral lateral pulvinar, RM is the rostral medial pulvinar and LGNd is the dorsal lateral geniculate nucleus.* ***B****. Tree shrews based on descriptions of Luppino et al., 1988, Lyon et al., 2003a, and Chomsung et al., 2008. Pd is the dorsal pulvinar, Pc is the central pulvinar, and Pv is the ventral pulvinar.* ***C.*** *connections of the pulvinar complex in galagos based on current results and Wong et al., 2009.* ***D****. Anthropoid primates based on descriptions in Stepniewska et al., 1999, and Stepniewska et al., 2000, and Kaas and Lyon, 2007. PM is the medial pulvinar, PL is the lateral pulvinar, PIp is the posterior inferior pulvinar, PIm is the medial inferior pulvinar, PIcm is the central medial inferior pulvinar, PIcl is the central lateral inferior pulvinar. The VGLUT2 staining pattern in PIp and PIcm is from unpublished results.*

consistent with this interpretation. For example, in case 09-34 (Fig. 3.6), an injection site (CTB) extending more caudally within the superior colliculus resulted in terminal label that was located more centrally within the lateral pulvinar than the terminal label from a more rostral superior colliculus injection (FR) (also see cases Fig. 3.9 vs. Figs. 3.10 and 3.11).

As in previous reports on galagos (Glendenning et al., 1975; Diamond et al., 1992; Wong et al., 2009), we did not find terminal label within the rostral part of the medial pulvinar. However, caudally, portions of our Pp cross the brachium dorsally into the territory of the classically defined superior pulvinar, a region more recently defined as the medial pulvinar (Wong et al., 2009). We did see terminal label in this more dorsomedial location, but only co-localized within our darkly staining VGLUT2 region of the caudal pulvinar within Pp. Rather than part of the medial pulvinar, comparisons with anthropoid primates indicate that Pp is part of the inferior pulvinar (PIp).

The inferior pulvinar of galagos, as described by Glendenning et al. (1975) has been subsequently divided into three domains based on cortical and tectal connections. A large central inferior pulvinar (IPc) located laterally, a medial inferior pulvinar (IPm) located medially, and a posterior pulvinar (IPp) located at the most posterior end of the pulvinar complex. Both IPc and IPm have connections with striate and extrastriate cortical areas (Symonds and Kaas, 1978; Wall et al., 1982; Wong et al., 2009), while IPp receives projections from the superior colliculus but has few connections with striate cortex (Glendenning et al., 1975; Symonds and Kaas, 1978; Diamond et al., 1992; Wong et al., 2009). We confirm that there are dense projections to the caudal portion of the

pulvinar as described by Glendenning et al., 1975, Diamond et al., 1992, and Wong et al., 2009, but, for the first time we are able to anatomically define the caudal tectal termination zone using VGLUT2 staining procedures. We also propose that this zone includes two separate subdivisions, which we call the posterior pulvinar (Pp) and the posterior central pulvinar (Ppc). Additionally, we found that Pp is not confined to the classically defined region of the inferior pulvinar, which lies below the brachium of the superior colliculus, but extends both above and below the brachium (See Fig. 3.6 sections 163 and 158, Fig. 3.7 section 143). In anthropoid primates, the results of recent studies indicate that several subdivisions of the inferior pulvinar also extend above the brachium of the superior colliculus (e.g. Stepniewska and Kaas, 1997).

PIm of galagos, as described by Symonds and Kaas (1978), and Wong et al., 2009, is located medially within the classically defined inferior pulvinar. In all cases in the present study, a patch of terminal label was located medially, but outside of the VGLUT2 densely staining posterior pulvinar. Likely this projection is to PIm as described previously in galagos. This division of the inferior pulvinar of galagos could correspond to PIm of anthropoid primates.

In summary, the pulvinar complex of galagos has more subdivisions than previously reported. Although the classically defined inferior pulvinar of galagos has been located below the brachium of the superior colliculus, the present study provides evidence that the inferior pulvinar does traverse above the brachium, with Pp extending more dorsally than subdivisions of the inferior pulvinar in anthropoid primates. This more dorsal location suggests a rotational shift in the location of subdivisions of the

inferior pulvinar occurred in anthropoid primates from the pulvinar complex of galagos and other mammals (see below), making homologous nuclei more difficult to identify.

3.5.2. Comparisons with other Euarchontoglires

The Euarchontoglires clade of placental mammals includes a number of species whose pulvinar organization has been well studied, including New and Old World monkeys, rodents, and tree shrews. Part of our goal in the current study was to compare the organization of the pulvinar complex in galagos and other members of the Euarchotoglire clade in order to reveal common features and specializations. Here we first describe the pulvinar organization in galagos and other primates, and then focus on the pulvinar organization in squirrels and tree shrews, non-primate members of the Euarchontoglires clade.

3.5.2.1. Pulvinar organization in primates

Pulvinar organization in primates is largely understood from the results of experimental studies in New and Old World monkeys (Fig 3.14D). Early studies divided the inferior pulvinar (PI) into three nuclei, a posterior nucleus, PIp, and a "central" inferior nucleus, PIc with inputs from the superior colliculus (Mathers, 1971; Lin and Kaas, 1979), and a medial inferior nucleus, PIm, with few, if any, inputs from the superior colliculus (Lin and Kaas, 1979, 1980). On the basis of marked architectonic differences, especially for brain sections processed for AChE, PIc has been subsequently divided into a smaller medial nucleus, PIcm, and a larger lateral nucleus, PIcl (Stepniewska et al., 1997). The lateral pulvinar, PL, extends ventrally along the lateral border of PIcl (See Fig 3.14D). Both PIcl and PL project topographically to V1 and V2 (Adams et al, 2000; Kennedy and Bullier, 1985), while PIp, PIm, and PIcm all project to

temporal cortex in the MT complex, MT, MST, MTc, and FST (See Kaas and Lyon, 2007 for review). PIcl and PL appear to contain the two large retinotopically organized representations of the contralateral visual hemifield that have been previously described (Allman and Kaas, 1972; Gattas et al, 1978; Bender, 1981; Ungerleider et al., 1983; Shipp, 2001). There may be other, less precise retinotopic representations in PIcm, PIm, and PIp, but this is not well established. The medial pulvinar, PM, is distinguished by projections to a number of non-visual as well as visual cortical regions (see Stepniewska, 2004; Jones, 2007 for review).

Our current interpretation of the relationship of the pulvinar subdivisions in galagos to those proposed in monkeys is that the large VGLUT2 positive region, Pp, in galagos is homologous to PIp of monkeys. Both regions occupy the most medial portion of the inferior pulvinar, while extending above the brachium of the superior colliculus into the traditional territory of the medial pulvinar (Stepniewska et al., 1997). Both regions receive dense inputs from the superior colliculus, and project to temporal visual cortex largely around MT (Kaas and Lyon, 2002; Wong et al, 2009). A major difference is that Pp extends into a more caudodorsal position in galagos, displacing PM laterally. The smaller Ppc region in galagos is similar to PIcm of monkeys in having superior colliculus inputs, and having a more lateral location within the medial aspect of the inferior pulvinar than Pp/PIp with a possible 'fusion zone' linking Pp/PIp and Ppc/PIcm (Stepniewska et al., 1999). Ppc may be a displaced part of Pp, or the homolog of PIcm of monkeys. If so, PIm of monkeys may occupy the small space, seen in some brain sections, between Pp and Ppc in galagos, or in tissue rostral to Ppc, or be poorly differentiated or absent. Much of the Pp-Ppc regions project to the MT complex and other

temporal visual areas in galagos (Glendenning et al., 1975; Raczkowski and Diamond, 1980; Wong et al., 2009). More lateral parts of the pulvinar complex in galagos (PI and PL) that project topographically to V1 (Symonds and Kaas, 1978; Wong et al., 2009), forming two adjoining topographic maps, likely corresponding to PL and PIcl of monkeys. Previous reports on New World monkeys have shown projections from the superior colliculus to PIcl and even PL (Stepniewska et al., 1999; 2000). However, in Old World monkeys only minor projections from the superior colliculus to PIcl and the most lateral aspect of PL have been reported (Stepniewska et al., 2000; Lyon et al., 2010). Therefore, projections from the superior colliculus to PIcl and PL divisions of the pulvinar could have been present within early primates, but are reduced in Old World monkeys.

A smaller dorsomedial region, PM, in galagos resembles PM of monkeys, but corresponding connections have not been reported.

3.5.2.2. Pulvinar organization in other mammals and homologies with primates

Our present view of pulvinar organization in galagos, given the differences in the arrangement of the inferior pulvinar nuclei from those in monkeys, invites further comparisons with non-primate mammals. Such a comparison is complicated by the early view that the pulvinar is a structure found only in primates, and the resulting tendency of subsequent investigators to refer to all or much of the pulvinar in non-primates as the lateral posterior nucleus or nuclei (see Kaas, 2007 and Jones, 2007 for review). More recently, it has become apparent that all mammals have a part of the visual thalamus with inputs from the superior colliculus and connections with visual cortex that can be reasonably called the pulvinar, but subdivisions homologous to those of the pulvinar in

primates have been difficult to identify (Jones et al., 2007; Wong et al., 2009; Manger et al., 2010; Baldwin et al., 2011). Here we consider the subdivisions of the pulvinar in two highly visual mammals of the Euarchontoglires clade, tree shrews and gray squirrels. Tree shrews (Scandentia) are of special interest as they are the closest living relatives of primates that have been studied (Kaas, 2002; Kaas, 2005). Squirrels and other rodents are also of great interest, as rodents constitute one of the major branches of the Euarchontoglires radiation (Murphy et al., 2001; Meredith et al., 2011).

In both squirrels and tree shrews, a caudal part of the pulvinar, known as the caudal pulvinar (C) in squirrels (Fig. 3.14A) and the dorsal pulvinar (Pd) in tree shrews (Fig 3.14B) get diffuse inputs from the superior colliculus and project to temporal visual cortex (Robson and Hall, 1977; Lyon et al., 2003; Wong et al., 2008; Chomsung et al., 2010). Both C and Pd stain darkly for AChE and for VGLUT2 (Lyon et al., 2003; Chomsung et al., 2008; Baldwin et al., 2011), and its dorsomedial position at the caudal pole of the pulvinar is in a location more similar to Pp in galagos than PIp in monkeys. We propose that the C nucleus in squirrels, Pd in tree shrews, Pp in galagos, and PIp in monkeys are homologous, but somewhat differently displaced, especially by the enlarged medial and lateral divisions of the pulvinar in monkeys and other anthropoid primates. Other mammals have a darkly staining AChE zone with superior colliculus inputs and projections to temporal visual cortex, as has been well studied in cats (Graybiel and Berson, 1980; Abramson and Chalupa, 1988; Berson and Graybiel, 1991; Hutsler and Chalupa, 1991). A homologous pulvinar nucleus may exist in all mammals, and even in reptiles and birds (see Fredes et al., 2012 for review).

A significant difference between the pulvinar complex of galagos and that of many non-primates is that, in diurnal rodents (Robson and Hall, 1977; Kuljis and Fernandez, 1982; Baldwin et al., 2010; Fredes et al., 2012), other diurnal mammals (Luppino et al., 1988), and even birds (Karten et al., 1997; Marin et al., 2003), the caudal nucleus receives inputs from both the ipsilateral and contralateral superior colliculus, while only the ipsilateral superior colliculus projections to the pulvinar were observed in galagos. This difference could reflect a specialization of the superior colliculus in primates where each colliculus represents only the contralateral visual hemifield from both eyes (Lane et al., 1973; Kaas and Preuss, 1993; May, 2006), while the complete retina of the contralateral eye is represented in the superior colliculus of non-primate mammals (Lane et al., 1971; Kaas, 2002 more Kaas et al., 1974; May, 2006). However, some reports in macaque monkeys have described bilateral projections to the inferior pulvinar (Benevento and Rezak, 1976; Trojanowski and Jacobson, 1975), and a lack of contralateral tectal projections to the pulvinar complex in nocturnal rodents (Donnelly et al., 1983; Cadusseau and Roger, 1985; Masterson et al., 2009), and rabbits (Graham and Berman, 1981) have also been reported. More studies are needed to further document those mammals with bilateral projections to the pulvinar and those with only ipsilateral projections. It will also be useful to determine if the contralateral projections to the pulvinar in non-primates arise from the rostral part of the superior colliculus, where the ipsilateral hemifield is represented.

We also suggest that Ppc in galagos is homologous with PIcm of monkeys. Alternatively, Ppc and Pp of galagos correspond with PIp of monkeys. Likewise, the caudal nucleus of squirrels and the dorsal pulvinar of tree shrews may correspond only to

Pp of galagos, or both Pp and Pc. Other homologies are less certain. The larger retinotopically organized nuclei that project to V1 and V2, RM and RL in squirrels and Pc and Pv in tree shrews, are likely homologous with PIcl and PL of primates with RL of squirrels and Pc in tree shrews most likely corresponding to PIcl. Thus, as many as four subdivisions of the visual pulvinar may have emerged in early stages of mammalian evolution.

3.5.3. Origins of the tectopulvinar pathway within the superior colliculus

Recently, Fredes et al., (2012) have revealed differences in the laminar origin of the tectal projections to the pulvinar in squirrels. Cells that project to the caudal pulvinar originate in the lower SGS, while projections to the rostral divisions of the pulvinar originate to in the upper SO of the superior colliculus. In our current study, we did not isolate injections within the SGS or the SO. However, recent insitu experiments conducted of Balaram et al. (2011) indicate that cells within the lower SGS of galagos express VGLUT2 mRNA, while cells within the SO do not. Thus, it is likely that the projections to the caudal divisions of the pulvinar originate from the SGS cells that express VGLUT2 mRNA. We are uncertain as to where the superior colliculus projections to more rostral locations in the pulvinar originate, but such projections could likely be from the upper SO, as the case with the more superficial injection in the superior colliculus (10-51: Fig. 3.7) showed dense projections to the caudal pulvinar but not to more rostral and lateral subdivisions. In addition, these rostral projections do not coincide with strong VGLUT2 staining, and cells within the SO of the superior colliculus do not express VGLUT2 mRNA. Previous reports in galagos have shown that only cells within the lower SGS (Raczkowski and Diamond, 1981) and cells bordering the SGS and

SO (Diamond et al., 1992) project to the pulvinar complex. However, since only a percentage of cells in the lower SGS express VGLUT2 mRNA, projections to the rostral divisions of the pulvinar could also originate from cells within the lower SGS that do not utilize VGLUT2.

3.6. REFERENCES

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CHAPTER 4

CORTICAL PROJECTIONS TO THE SUPEROIR COLLICULUS PROSIMIAN GALAGOS (*OTOLEMUR GARNETTI*) [3]

4.1. ABSTRACT

The superior colliculus (SC) is a key structure within the extrageniculate pathway of visual information to cortex, and is highly involved in visuomotor functions. Previous studies in anthropoid primates have shown that superficial layers of the SC receive direct inputs from various visual cortical areas such as V1, V2, and MT, while deeper layers receive direct inputs from visuomotor cortical areas within the posterior parietal cortex, and the frontal eye fields. Very little is known, however, about the corticotectal projections in prosimian primates. In the current study, we investigated the sources of cortical inputs to the SC in prosimian galagos (*Otolemur garnetti*) using retrograde anatomical tracers placed into the SC. The superficial layers of the SC in galagos received the majority of their inputs from early visual areas, and visual areas within the MT complex. Yet, surprisingly, MT itself had relatively few corticotectal projections. Deeper layers of the SC received direct projections from visuomotor areas including the posterior parietal cortex, and premotor cortex. However, relatively few corticotectal projections originated within the frontal eye fields. While prosimian galagos resemble other primates in having early visual areas project to the superficial layers of the SC, with

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higher visuomotor regions projecting to deeper layers, the results suggest that MT and FEF projections to the SC were sparse in early primates, remained sparse in present day prosimian primates, and became more pronounced in anthropoid primates.

4.2. INTRODUCTION

The present study is part of an extended effort to compare and contrast the organization of visual systems across members of the two major branches of the primate radiation, the prosimians and the anthropoids. The cortical distributions of neurons projecting to the superior colliculus has been studied in both New World monkeys (Cusick, 1988; Collins et al., 2005), and Old World monkeys (Fries et al., 1984, 1985; Lock et al., 2003), but not in any prosimians. In both New World and Old World monkeys, early visual areas, including the first, second, and third visual areas (V1, V2, and V3 respectively), as well as the middle temporal visual area (MT) project densely to the superficial layers of the superior colliculus, while visuomotor areas of posterior parietal cortex and frontal cortex, in particular, the frontal eye fields (FEF), project densely to the deeper layers. Additionally, projections from parts of prefrontal cortex, in addition to the FEF, have been reported in macaques and Cebus monkeys (Goldman and Nauta, 1976; Leichnetz et al., 1981; Fries, 1984, 1985; Johnston and Everling, 2006, 2009; Pouget et al., 2009). There are few cortical projections from auditory, somatosensory, higher order multisensory, motor, and cingulate areas to the superior colliculus.

The main goal of the current study was to determine corticotectal projections to the superior colliculus in prosimian galagos and to relate our findings to those from simian primates. Extant prosimian primates more closely resemble early primates in brain size relative to body size than do anthropoid primates (Le Gros Clark 1931; Radinsky, 1975; Stephan et al., 1981; Jerison, 2007), and their cortical organization may more clearly reflect that of the early ancestors of all primates (Kaas, 2007). Comparing data

from prosimian galagos to information from anthropoids, therefore, provides a means of identifying brain features that are either shared with anthropoid primates, or are possibly specializations of one or the other main branches of the primate radiation.

Cortical organization has been extensively studied in prosimian galagos and many of the cortical areas with projections to the superior colliculus in anthropoid primates have been anatomically and physiologically described in galagos. For instance, subdivisions of frontal cortex have been defined, including the frontal eye fields (Wu et al., 2000), as have sensorimotor regions of posterior parietal cortex (Stepniewska et al., 2005, 2009a,b). Additionally, recent evidence has shed light on the organization of some of the early visual areas, especially V3 (Lyon and Kaas, 2002), and areas that are part of the MT complex (Kaskan and Kaas, 2007). Yet, little is known about the connections of these areas with the superior colliculus.

In the present study, afferent connections to the superior colliculus in galagos were studied by injections of retrograde anatomical tracers into the superior colliculus. We were able to examine differences in the distributions of corticotectal neurons labeled by superficial and deep injections, and by injections into different topographic locations in the superior colliculus. The results revealed some expected similarities in the distributions of corticotectal projections in primates. Most notably, projections to the superficial layers of the superior colliculus were largely from early visual areas. The locations of labeled corticotectal neurons were primarily in topographic locations within the visual areas that matched those of the injection sites. In addition, single injections in the superior colliculus labeled patches of neurons at several locations in temporal visual cortex, a region where the functional subdivisions are poorly

understood in galagos. Unexpectedly, only a few corticotectal projections originated from MT and injections of tracers in locations that included the deeper layers of the superior colliculus labeled few neurons in the locations of FEF.

4.3. MATERIALS AND METHODS

In order to reveal distributions of corticotectal projections, 5 adult galagos (*Otolemur garnetti)* received injections of retrogradely transported tracers in the superior colliculus. All surgical procedures were in accordance with an approved protocol under the Vanderbilt University Institutional Animal Care and Use committee and followed guidelines published by the National Institute of Health. Injections placed in the superior colliculus labeled neurons in neocortex, which were plotted and related to cortical areas.

4.3.1. Surgical procedures and injections

Surgical procedures were similar to those of Baldwin et al. (2011). In brief, galagos were initially anesthetized with an intramuscular (IM) injection of ketamine hydrochloride (120 mg/kg), and maintained under anesthesia using isoflurane anesthesia (1-3%) through a tracheal tube. Lidocaine was placed in both ears as well as along the midline of the scalp, and heads were held in a stereotaxic frame. Heart rate, O_2, CO_2 levels, and body temperature were monitored and recorded throughout the surgery. The scalp was cut along the midline and retracted to expose the skull. A craniotomy over the left parietal and occipital lobes was placed to expose the caudal half of the intraparietal sulcus and the medial occipital lobe. The dura was reflected, and enough caudomedial cortex was removed by aspiration to allow the medial surface of the left superior colliculus to be visualized (Fig. 4.1). The right hemisphere was then retracted slightly to expose the medial aspect of the right superior colliculus (Fig. 4.1C). Then 0.2-0.75µl of cholera toxin B-subunit (CTB: Molecular Probes Invitrogen, Carlsbad, CA; 10% in distilled water) and/or 0.2-0.75 µl of fluoro ruby (FR: Molecular Probes Invitrogen; 10% in 0.1M phosphate buffer) were pressure injected at separate sites into the medial portion

of the superior colliculus, using a Hamilton syringe outfitted with a beveled glass pipette tip. Any leakage of tracer to the superior colliculus surface during injections was removed with sterile saline flushes to prevent tracer contamination of surrounding brain tissue. After injections, gelfoam was placed into the lesion site, gelfilm was then placed over the brain, the craniotomy was closed using dental cement, and the scalp was sutured. Animals were then taken off anesthesia, given Buprenex (0.3mg/kg IM) for analgesic, and monitored during recovery.

In once case, 09-34, 7 days after the initial surgery, we conducted a microstimulation experiment within the right frontal cortex to identify the frontal eye field (FEF). In this case, the galago was anesthetized with an intramuscular IM injection of ketamine hydrochloride (120mg/kg) and during surgical procedures was maintained under anesthesia using isofluorane (1-3%). For the microstimulation session the anesthesia was changed to a mixture of ketamine hydrochloride (30-60mg/kg/hr intravenously) and xylazine (0.4mg/kg IM). A craniotomy was placed over the frontal lobe to expose the frontal sulci (FS). A low-impedance tungsten microelectrode (1.0 MΩ) was mounted on an electrode holder and oriented perpendicular to the cortical surface. The electrode was then lowered into the brain anterior and ventral to the FS, and monophasic pulses of 0.2msec of electrical current were delivered in 60-msec trains at 300Hz. The FEF was identified as the region of the cortex that elicited eye movements during stimulation. Most eye movements were elicited with thresholds of 65-150 μA. The mapping session was kept short (5-6 hours) to minimize damage to the cortex. After the FEF was mapped, 4 electrolytic lesions were placed along its borders and then the galago was sacrificed with an overdose of sodium pentobarbital.

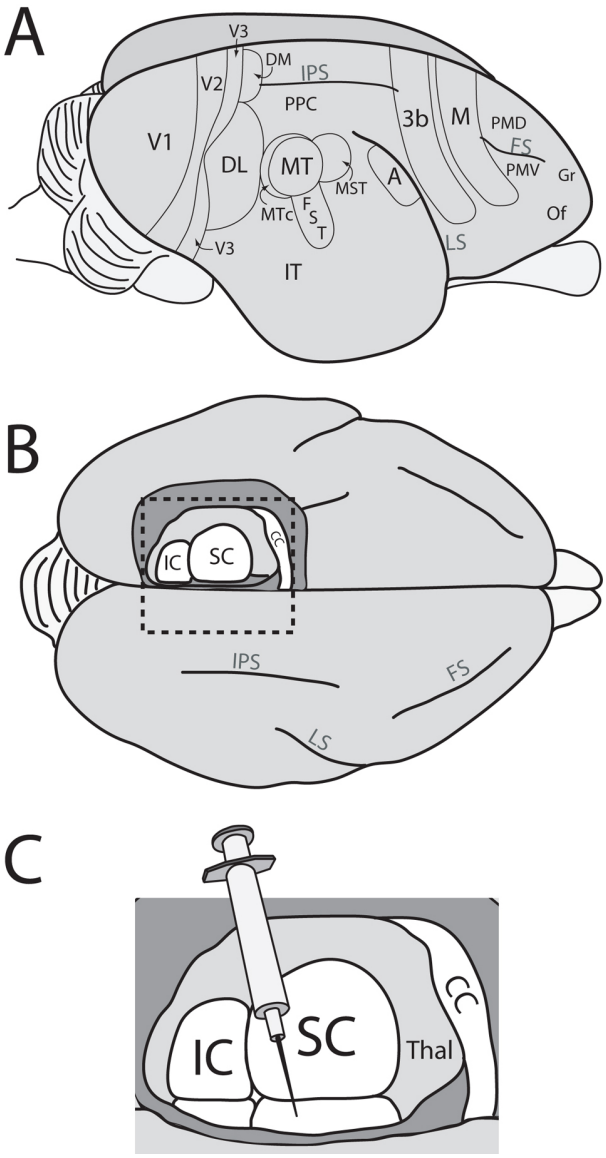

Figure 4.1. Cortical organization of prosimian galagos and tracer injection methods. A: The current understanding of cortical organization of prosimian galagos based on Fang et al. (2005) and Kaas and Lyon (2002). Areas include primary visual area (V1), secondary visual areas (V2), the third visual area (V3), dorsomedial area (DM), dorsolateral area (DL), middle temporal area (MT), middle temporal crescent (MTc), medial superior temporal sulcus (MST), the fundus of the superior temporal sulcus (FST), inferior temporal cortex (IT), auditory cortex (A), primary somatosensory cortex (3b/S1), motor cortex (M), premotor dorsal area (PMD), premotor ventral area (PMV), orbital frontal cortex (Of), and the granular frontal cortex (Gr). LS is the lateral sulcus, IPS is the intraparietal sulcus, and FS indicates the frontal sulci. B: A dorsal view of the galago brain showing the location of the lesion in the left hemisphere in order to access the superior colliculus for injections. C: A close-up view of the aspirated cortex with a view of the superior colliculus. The right hemisphere was retracted slightly in order to view and inject the right superior colliculus.

4.3.2. Tissue processing and data analysis

After 5-7 days of survival, galagos were sacrificed using an overdose of sodium pentobarbital (80 mg/kg) and perfused with phosphate buffered saline (PBS: pH 7.4) followed by 2% paraformaldehyde, and finally with a solution of 2% paraformaldehyde with 10% sucrose. The brains were removed, the cortex was separated from underlying brain structures, the sulci were opened and the brain was flattened as described in Krubtizer and Kaas (1990). Both cortex and thalamus with brainstem were placed in 30% sucrose solutions at 4^{o}C for 20-48 hours. The right flattened cortex was cut parallel to the cortical surface on a freezing microtome at a thickness of 40µm, while the brainstem was cut coronally at a thickness of 40µm. Alternating sections were processed to reveal tracer label and cortical architecture. The cortical sections were divided into four series and processed for cytochrome oxidase (CO: Wong-Wiley, 1979), myelin (Gallyas, 1979), or CTB using a protocol described in Baldwin and colleagues (2011). The last series was not processed, but instead mounted directly onto slides and coverslipped for fluorescent analysis of neurons labeled with FR. The brainstem was processed in five series, which were CO, CTB, FR, and acetylcholinesterase (AChE: Geneser-Jensen and Blackstand, 1971); the fifth series was set aside and processed as part of another study. Here, we describe only the results from cortex and the locations of injections in the superior colliculus.

To determine the locations of retrogradely labeled cells, CTB and FL sections were plotted using a Neurolucida system (MicroBrightField, Williston, VT).

Brainstem sections stained for CO and AChE were used to identify the layers of the superior colliculus. Injection sites were illustrated on dorsal views of the superior

colliculus surface, which were reconstructed from coronal brain sections and matched with photographs of the superior colliculus taken during brain dissection. Cortical sections plotted for labeled neurons were aligned with adjacent sections stained for cortical architecture using common blood vessels and other features. Alignments were made locally in order to most accurately relate plotted neurons to architectonic fields. The borders of some cortical areas (V1, V2, MT, 3b, A1) were determined architectonically using CO or myelin stained sections (see below), or were estimated based on locations and relationships to sulci and other cortical areas determined in previous studies on galago cortical organization. Finally, numbers of cells within cortical areas were counted in Adobe Illustrator, using Document Info settings. Photographs of tissue sections were taken using a DMX1200F digital camera mounted to a Nikon E800S microscope (Nikon Inc., Melville, NY). Photomicrographs were adjusted for brightness and contrast using Adobe Photoshop, but were otherwise not altered.

4.3.3. Locations of injection sites

Injection sites and tracer spread were related to the layers of the superior colliculus identified in CO and AChE stained sections (Fig. 4.2), and to the representation of the contralateral visual hemifield in the superior colliculus. The superior colliculus has 7 main layers (May, 2006), which include the stratum zonale (SZ), the stratum griseum superficiale (SGS), stratum opticum (SO), stratum griseum intermediale (SGI), stratum album intermediale (SAI), stratum griseum profundum (SGP), and stratum album profundum (SAP) (Fig. 4.2). Though the SGS and SGI have been subdivided further (Balaram et al., 2011), we do not subdivide these layers in the current study. SZ, SGS, and SO are parts of the superficial SC, which has been primarily associated with visual

sensory functions. The intermediate (SGI and SAI) and deep (SGP and SAP) layers have been attributed to sensorimotor integration and various motor functions.

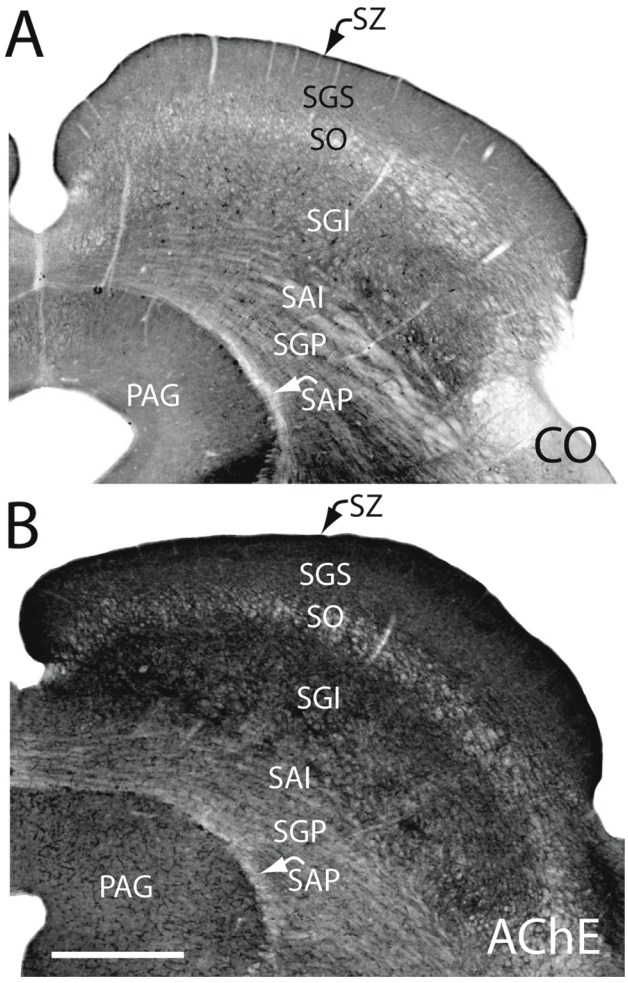

*Figure 4.2. Coronal sections of the superior colliculus stained for cytochrome oxidase (CO: **A**) or acetylcholinesterase (AChE: **B**). Photographs are taken from two different galagos. SZ, stratum zonale; SGS, stratum griseum superficiale; SO, stratum opticum; SGI, stratum griseum intermediale; SAI, stratum album intermediale; SGP, stratum griseum profundum; SAP, stratum album profundum. Medial is left. Scale bar . 1 mm.*

Superior colliculus injections that involved SZ, SGS, SO, and the most superficial aspect of the SGI are described in our superficial and intermediate injection section cases. These types of injections were observed for cases 07-105, 07-111, and 08-40. For all of these cases the injection site cores were within the SGS, but the injection site spread

included SGS, SO, and the upper aspect of the SGI. Though injection sites did include portions of the upper SGI in these cases, for simplicity, we listed and grouped such injections in our results section as 'superficial' injections. Cases 09-03 and 09-34 had superior colliculus injection sites that involved layers below the SGI and are therefore grouped into our results section describing deep layer injections; however it is important to note that these cases had tracer spread into the superficial layers of the superior colliculus.

The visuotopic organization of the superior colliculus in galagos has been determined using microelectrode recording experiments (Lane et al., 1973). The lateral superior colliculus represents the lower visual field while the upper visual field is represented medially; peripheral vision is represented caudally, and central vision rostrally. All topographic determinations of injection site locations were based on estimates, after dorsal reconstructions of the injection site, and by comparing such locations with the topographic maps described in Lane et al. (1973). Most of our injections were within the representation of the upper visual field in the superior colliculus, and only one CTB injection included representations of both upper and lower visual fields.

Overlying cortical tissue was analyzed for possible tracer contamination both during brain dissection as well as within our processed tissue sections. We did not notice signs of cortical contamination, and believe that our results reflect corticotectal connections and not cortico-cortico connections.

4.3.4. Identification of cortical areas

Cortical areas examined in this study within the occipital cortex are V1, V2, V3, DM and DL (Fig. 4.1A). Area V1 was reliably identified in galagos by its charactertistic CO blob-interblob pattern of staining and dark myelination (Condo and Casagrande, 1990; Kaskan and Kaas, 2007; Wong and Kaas, 2010). V2, rostral to V1, stains less darkly for myelin and, unlike anthropoid primates, has only a weak stripe-like pattern of CO staining at best (Condo and Casagrande, 1990; Kaskan and Kaas, 2007; Wong and Kaas, 2010). Thus, the rostral border of V2 was estimated by its known width. The width of V2 is variable across its length measuring 1mm near central vision and extending out to 2 to 3mm wide at peripheral positions (Rosa et al., 1997; Collins et al., 2001). Determining the borders of V3 was difficult because of the lack of clear anatomical landmarks; however, V3 stains moderately for myelin and darkly for CO and is located along the rostral border of V2. V3's width varies along its length, but it can be as wide as 2mm in the periphery (Lyon and Kaas, 2002; Wong and Kaas, 2010). DM is located along the rostral border of dorsal V3 and stains moderately for myelin (Beck and Kaas, 1998; Krubitzer and Kaas, 1990; Wong and Kaas, 2010), but more so than bordering areas, except for V3. The full mediolateral extent of DL (V4) is uncertain, therefore we designated the cortical region between V3, MTc, IT, and DM as DL (Fig. 4.1A). Within V1, V2, V3, and DL, the upper visual hemifield is represented ventrally and the lower visual hemifield is represented dorsally (Rosa et al., 1997; Lyon and Kaas, 2002). The precise topographic organization of DM is not fully known, but DM represents both the upper and lower visual hemifields (Lyon and Kaas, 2002).

Temporal cortex was divided into the following areas: MT, MTc, MST, and FST, IT, and the auditory region (Aud). MT can be identified from surrounding cortical areas by its characteristic dense myelination and heavy CO staining relative to surrounding cortex (Allman et al., 1973; Symonds and Kaas, 1978; Wall et al., 1982; Krubitzer and Kaas 1990; Collins et al., 2001; Kaskan and Kaas, 2007; Wong and Kaas, 2010). Within MT, the upper visual field is represented ventrally, and the lower visual field is represented dorsally with central vision caudal and peripheral vision rostral (Allman et al., 1973). The medial superior temporal area, MST, is located just rostral to MT and stains darkly for CO and moderately for myelin (Maunsell and Van Essen, 1983; Weller and Kaas, 1984; Krubitzer and Kaas, 1990; Wong and Kaas, 2010). The fundal area of the superior temporal sulcus, FST, is just ventral to MT. As galagos do not have a superior temporal sulcus, FST is on the cortical surface. FST stains moderately for myelin and CO (Krubitzer and Kaas, 1990; Kaskan and Kaas, 2007; Wong and Kaas, 2010). This area has been divided into ventral, FSTv, and dorsal, FSTd, divisions (Kaas and Morel, 1993); however, we do not distinguish divisions in the present report. The topographic organizations of FST and MST are not well understood but it is thought that MT and MST have adjoining representations of peripheral vision. MTc is distinguishable as a narrow strip of cortex that borders dorsal, caudal, and ventral portions of MT and stains heterogeneously for myelin and CO (Kaas and Morel 1993; Tootell et al., 1985; Kaskan and Kaas, 2007; Wong and Kaas, 2010). We defined IT as the region of cortex ventral to the MT complex, rostral to DL, and caudal to auditory cortex including space for proposed belt and parabelt auditory cortex (Fig. 4.1A). There are likely several cortical areas within IT (Zilles et al., 1979; Preuss and Goldman-Rakic, 1991a; Wong and

Kaas, 2010) but for simplicity, we present our results with respect to a single IT region. Finally, an auditory primary-like region was identified as a darkly stained region in myelin and CO preparations, spanning the caudal bank and lip of the lateral sulcus (Brugge, 1982; Wong and Kaas, 2010).

Parietal cortex includes the primary somatosensory area (3b/S1), posterior parietal cortex (PPC), and the parietal ventral/secondary somatosensory region (PV/S2). We defined posterior parietal cortex as the region of cortex surrounding the IPS, rostral to DM, caudal to 3b/S1, and dorsal to MT, MST, and MTc (Fig. 4.1A). 3b/S1, was easily identified by it's dense CO, and myelin staining pattern (Wu and Kaas, 2003). PV/S2 lies along the rostral bank of the lateral sulcus and stains moderately for CO and myelin (Wu and Kaas, 2003; Wong and Kaas, 2010).

Primary motor cortex is rostral to somatosensory cortex and is characterized by moderate myelin and CO staining (Wong and Kaas, 2010). Dorsal premotor cortex (PMD) and ventral premotor cortex (PMV) were demarcated as the regions of frontal cortex rostral to motor cortex and either dorsal or ventral to the frontal sulci. FEF was determined in one case physiologically, and was placed in other cases in its expected location, slightly dorsal to the rostral tip to the frontal sulci (Wu et al., 2000). Finally, based on results of Wong et al. (2010), granular frontal (Gr) cortex was defined as the region of cortex rostral to the frontal sulcus dorsally, and orbitofrontal cortex was located ventral and rostral to the ventral premotor cortex.

4.4. RESULTS

Cortical projections to the superior colliculus were studied in 5 galagos. In three of these cases injections involved only the superficial and intermediate layers (07-105, 07-111, 08-40) and in the other two cases injections involved superficial, intermediate, and deep layers (cases 09-34 and 09-03). Most injections included the upper visual quadrant, and one case involved both the upper and lower visual fields. More superficial injections within the superior colliculus labeled neurons in visual areas of the occipital and temporal cortex, while injections involving deeper layers also labeled neurons in frontal and posterior parietal cortex. A summary of the number and percentage of labeled neurons from different cortical areas and regions to the superior colliculus is presented in Table 4.1.

Though it's difficult to determine the layers in which labeled neurons were located in the brain sections cut parallel to the cortical surface, the labeled cells were always found in the deeper sections for all cortical areas, and were judged to be mainly or exclusively in infragranular layers below layer 4. In V1 layer 4 stains especially darkly for CO (Wong and Kaas, 2010), and was easily identified in our sections. Labeled cells were found in sections below layer 4.

4.4.1. Cases with injections into the superficial layers of the superior colliculus

In galago 07-111 (Fig. 4.3) a fluoro-ruby (FR) injection was placed in the superior colliculus representing the upper visual quadrant within 15-45^{o} of paracentral vision. The injection site included the SZ, SGS, and SO layers of the superior colliculus (Fig. 4.2). Within V1, labeled neurons were mainly within the lateral part representing paracentral

Case #	Tracer	Inj Depth	# cells in visual areas																		
			Occipital Cortex					Temporal Cortex						Parietal Cortex			Frontal Cortex				Other	Total
			V1	V2	V3	DM	DL	MT	MTc	MST	FST	IT	Aud	PPC	S2/PV	3b/S1	M	FEF	PMD	PMV		# cells
07_105	FR	S and I	1049	196	13	11	20	0	0	3	34	39	3	3	0	0	4	NA	22	0	29	1426
	%#		73.6	14	0.9	0.8	1.4	0	0	0.2	2.4	2.7	0.2	0.21	0	0	0.3	NA	1.5	0	2.03	
	CTB	S	667	65	64	0	75	30	0	59	113	486	13	19	5	1	1	NA	3	0	59	1660
	%#		40.2	3.9	3.9	0	4.5	2	0	3.6	6.8	29	0.8	1.14	0.3	0.06	0.1	NA	0.2	0	3.55	
07_111	FR	S	1336	76	42	48	69	0	0	0	85	34	2	12	0	0	0	NA	7	2	35	1748
	%#		76.4	4.3	2.4	2.7	3.9	0	0	0	4.9	1.9	0.1	0.69	0	0	0	NA	0.4	0.114	2	
08_40	CTB	S and I	1343	99	73	0	174	21	0	61	157	204	3	5	0	0	0	NA	5	0	343	2488
	%#		54	4	2.9	0	7	1	0	2.5	6.3	8.2	0.1	0.2	0	0	0	NA	0.2	0	13.8	
09_03		S, I, & D	1810	1340	524	807	1044	13	N/A	34	510	1020	73	5361	187	15	37	NA	1411	1182	1970	17338
	%#		10.4	7.7	3	4.7	6	0	0	0.2	2.9	5.9	0.4	30.9	1.08	0.09	0.2	NA	8.1	6.817	11.4	
09_34	CTB	S, I, & D	6912	2586	2566	455	844	4	0	58	125	1473	0	2736	9	0	0	227	472	1003	235	19705
	%#		35.1	13	13	2.3	4.3	0	0	0.3	0.6	7.5	0	13.9	0.05	0	0	1.2	2.4	5.09	1.19	
	FR	S, I & D	3297	862	203	22	170	2	0	11	166	130	0	117	0	1	0	9	30	67	8	5095
	%#		64.7	17	4	0.4	3.3	0	0	0.2	3.3	2.6	0	2.3	0	0.02	0	0.2	0.6	1.315	0.16	

Table 4.1. Percentage and numbers of cells projecting to the superior colliculus of galagos from various cortical regions and areas.

vision of the upper quadrant, matching the retinotopic location of the injection in the superior colliculus. There was no clear relation between the locations of labeled cells and cytochrome oxidase blob and interblob regions. The pattern of labeled neurons in rows in lateral V1 in Fig. 4.3 is a consequence of imperfect flattening of V1 (area 17), as it was unfolded along its lateral margins. This resulted in individual sections moving between layers 4, without labeled cells, and 5, with labeled cells, multiple times along the extent of V1, and therefore creating the stripe like patterns. As adjacent sections were processed for histology, the gaps in the distribution of labeled neurons in V1 were not filled in this reconstruction.

In V2, labeled cells were concentrated in a single patch located laterally and next to V3 where paracentral vision of the upper quadrant near the vertical meridian is represented. A scattering of labeled cells was found in lateral V3. Two patches of labeled cells in DM were observed and are consistent with the evidence that DM represents the upper as well as the lower visual quadrant (Lyon and Kaas, 2002). Surprisingly, no labeled cells were found in MT or MST, while many of labeled neurons were found in

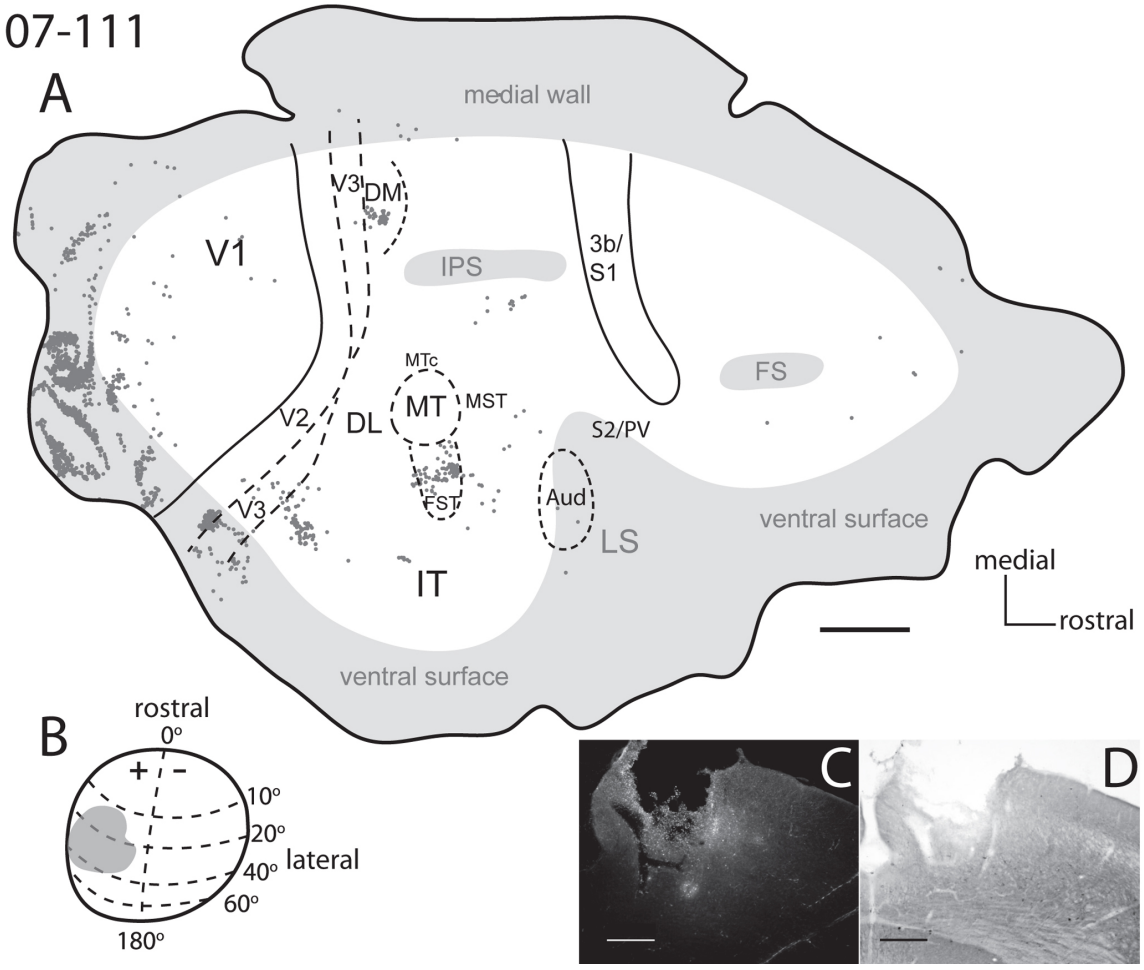

Figure 4.3. Cortical projections to the superficial and intermediate layers of the superior colliculus (SC) in case 07-111. A: The distribution of retrogradely labeled cells within flattened cortex after a fluoro-ruby (FR) injection into the SC. Gray dots represent FR cells. Solid lines represent borders determined using CO or myelin stains while dashed lines are estimated borders based on measurements and locations relative to other landmarks. The gray shaded area is cortex that was within sulci or along the medial or ventral surfaces. B: Dorsal view of the SC with the location of the FR injection site. C: Photomicrograph of the injection site within the SC. D: Photomicrograph of the adjacent section stained for CO. Scale bars . 5 mm in A; 0.5 mm in C,D.

FST. A small number of labeled cells were present in IT cortex ventral and rostral to FST. A few labeled cells were also present in posterior parietal cortex ventral to the IPS and between FST and auditory cortex. No labeled cells were found in somatosensory

cortex, insular cortex, cingulate cortex, and retrosplenial cortex, or (except for an occasional neurons) frontal cortex.

Similar results were obtained in galago 08-40 (Fig. 4.4). The injection site of this case was placed in the most caudal part of the medial superior colliculus, which represents peripheral vision of the upper visual quadrant (Lane et al., 1973). The injection involved the superficial layers of the superior colliculus, but was slightly deeper than the injection in case 07-111, and may have included the upper portion of the SGI. As expected from the location of this injection, labeled neurons were almost completely absent from portions of V1, V2, and V3 that represent central and even paracentral vision. The distributions of labeled cells within lateral V1, V2, and V3 were clearly in portions that represent the periphery of the upper visual field, and most of the label in medial V1 and V2 could be in parts that were unfolded from the calcarine sulcus when flattening cortex, and possibly in cortex that represents the upper visual quadrant (Rosa et al., 1997). Again, patches of labeled cells appeared in medial DM suggesting that peripheral vision of the upper visual quadrant is represented medially in DM (Rosa et al., 1997; Allman and Kaas, 1975). Another possibility is that these cells are within area M (Allman and Kaas 1976; Krubtizer and Kaas 1993), which also has both upper and lower visual field representations. A few labeled neurons were in lateral DL (V4) or in cortex just lateral to DL. Only a few labeled cells were in MT, while dense patches of labeled cells were in FST and MST, providing evidence that peripheral vision is well represented in FST and MST. Other concentrations of labeled cells were in several locations across the temporal lobe including regions medial and rostral to MST and FST. These results suggest that peripheral vision is represented in several locations in the inferior temporal

lobe. There were no foci of labeled neurons in visuomotor areas of posterior parietal cortex, motor and visuomotor areas of the frontal lobe, auditory cortex, or somatosensory cortex. These findings are consistent with previous evidence that projections of the superficial layers of the superior colliculus are from visual areas of cortex.

Our third case, 07-105, had CTB and FR injections into the superficial layers of the superior colliculus, with the FR injection being slightly more rostral than the CTB injection (Fig. 4.5B). The CTB injection was centered within the ventral portion of the SGS, but the tracer spread included the SZ, SGS, and the SO layers. The FR injection was slightly deeper within the superior colliculus with the injection core bordering the SO and ventral SGS, and the tracer spread including the SZ, SGS, SO and the dorsal portion of the SGI layers.

For the most part, clusters of CTB and FR labeled cells in cortex overlapped, with the distribution of FR labeled neurons slightly closer to the representation of central vision within V1 than the distribution of CTB labeled cells. Again, labeled cells were found in both lateral and medial V1 of the calcarine fissure. CTB and FR labeled cells were found in lateral V2, with the distribution of FR cells slightly displaced medially toward central vision. The CTB cells were closer to the representation of the horizontal meridian along the estimated V2/V3 border. A small patch of FR cells laid rostral to the CTB cells within V3. Unexpectedly, a few CTB labeled cells were present in dorsal V3 and in dorsal V2, while only FR cells were present in the location of DM. A few CTB labeled cells were within MT, but no FR cells. The bulk of labeled cells within the MT complex were at the ventral junction with MST, and dorsomedial FST. There was a high

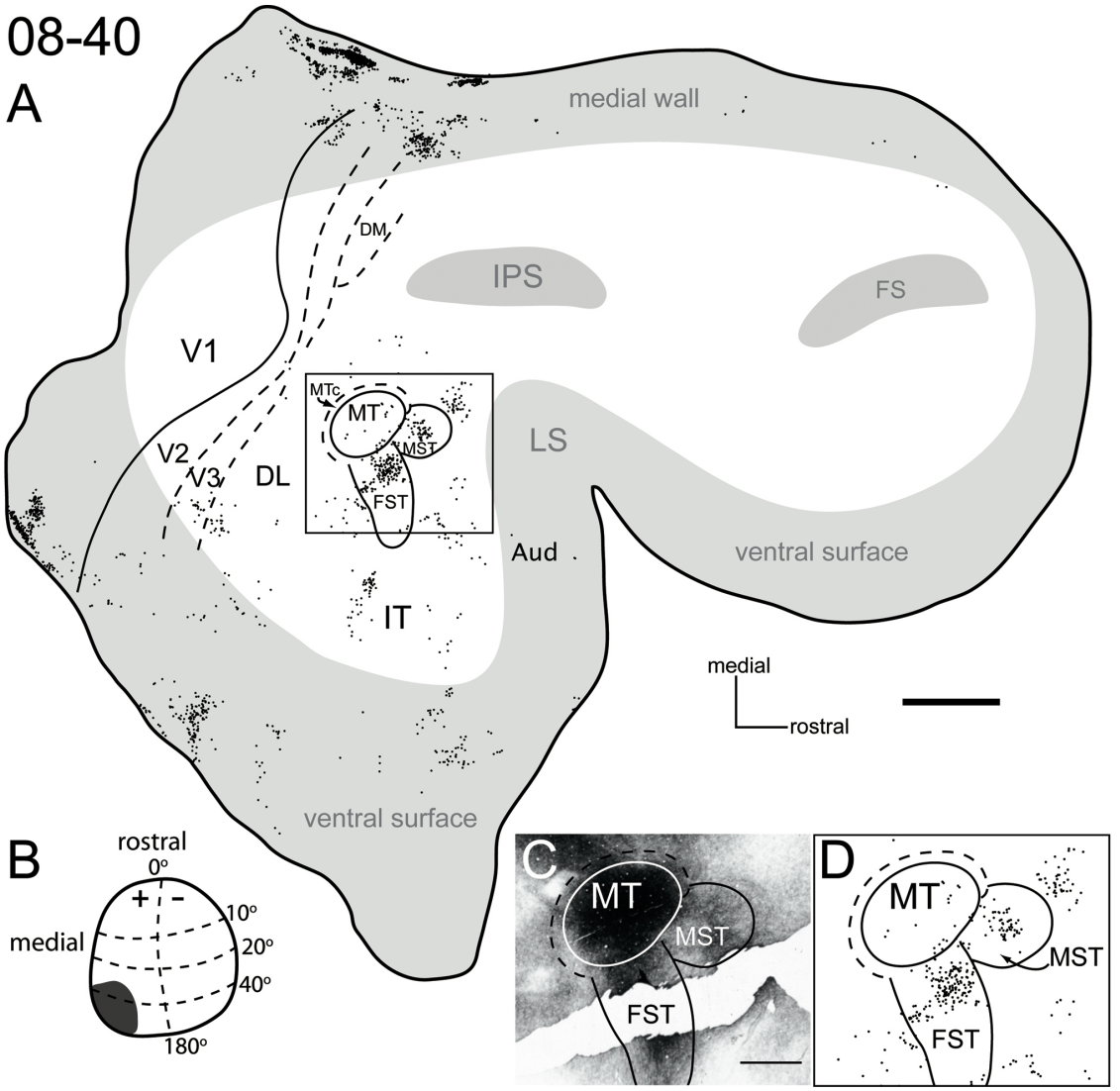

Figure 4.4. Cortical projections to the superficial and intermediate layers of the SC in case 08-40. A: Reconstruction of CTB-labeled cells in flattened cortex. B: Dorsal view of the injection site within the SC. C: Photomicrograph of a section stained for myelin indicating the location of MT, MST, MTc, and FST. D: Close-up view of the labeled cells within the MT complex region. Scale bars . 5 mm in A; 2 mm in C,D.

degree of overlap between the dense clusters of CTB and FR labeled cells within dorsal FST, with the FR cells located more rostrally within FST than the CTB labeled cells. Overlapping patches of FR and CTB labeled cells were present within lateral DL. Few FR cells were located within IT, with one dense patch of cells just ventral to the estimated FST border, while CTB labeled cells were found abundantly in IT with

multiple clusters throughout the region. CTB and FR cells were also located between auditory cortex and FST and MST, similar to case 08-40 (Fig. 4.4). There were a few cells within auditory cortex as well as cortex within the lateral sulcus. The deeper FR injection labeled a few cells within or medial to the frontal sulci (FS). No labeled cells were present within primary motor, ventral prefrontal cortex, and primary somatosensory cortex. A few labeled cells were within the region of the secondary somatosensory area and parietal ventral somatosensory (S2/PV) area.

4.4.1. Cases with injections into the deep layers of the superior colliculus

Case 09-34 had both CTB, and FR injections into the superior colliculus. The injection cores were again within the SO, but the tracer spread for both tracers included the SZ, SGS, SO, SGI, and into the SAI, as well as a small portion ventrally within the SGP (Fig. 4.6 E-H). The FR rostromedial injection was confined to the upper visual field representation. The more extensive CTB injection was largely caudal to the FR injection and included both upper and lower visual field representations within the superior colliculus. The pattern of labeled cells within early visual areas reflects the position of the FR and CTB injection sites with CTB labeled cells in both upper and lower field representations within V1, V2, V3, and DL, while labeled cells were absent in the central vision representations. FR labeled cells were found almost exclusively within upper field locations. Additionally, within the upper visual field representations of the early visual areas, the majority of FR labeled cells were more medially located and closer to the representation of central vision than most of the CTB labeled cells. Again, labeled cells formed lines within unfolded parts of striate cortex, and this is most likely due to uneven flattening. Consequently, the lines of cells observed in Fig. 4.6 are a result of brain

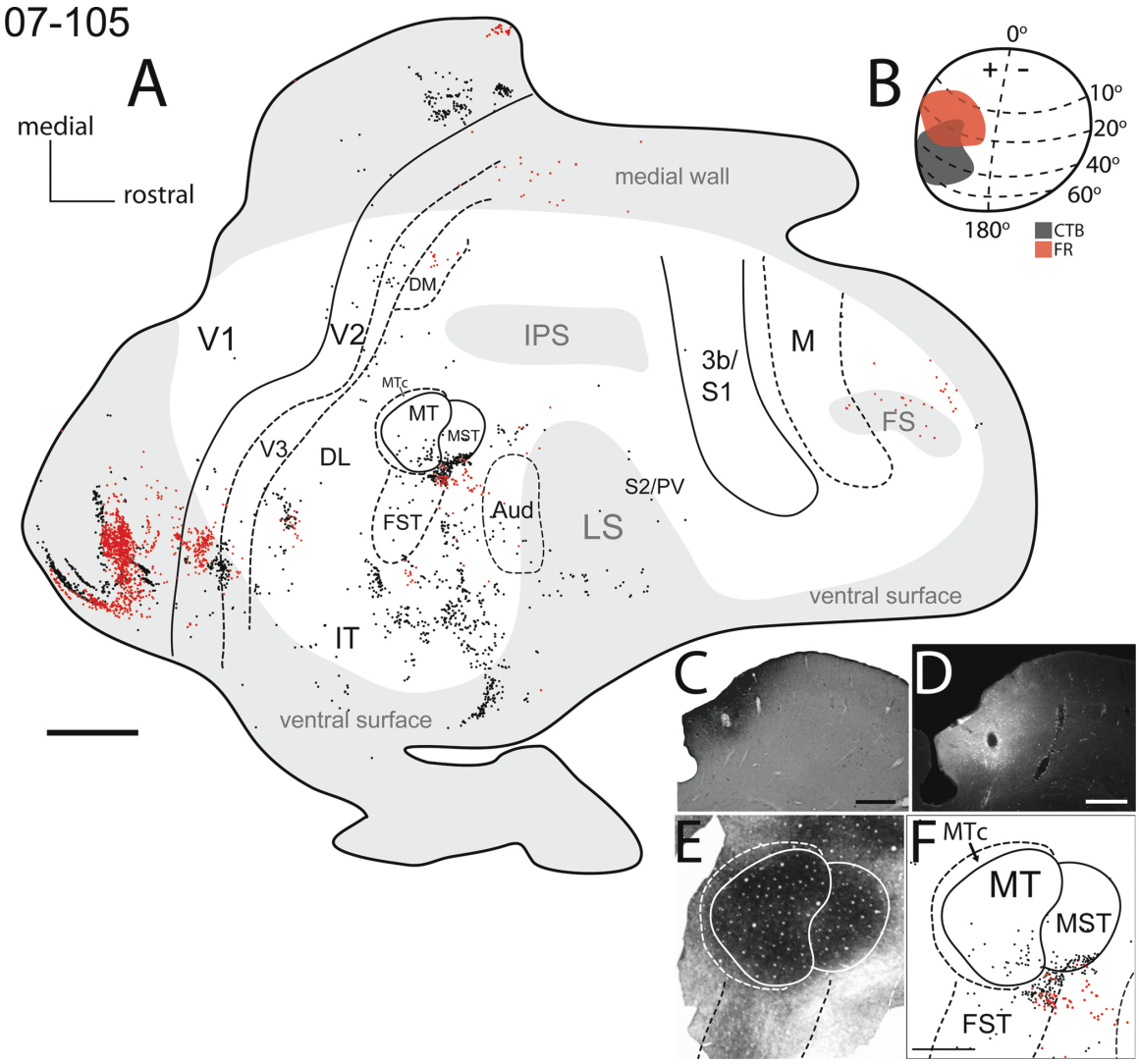

Figure 4.5. Cortical projections to the superficial and intermediate layers of the SC in case 07-105. A: The distribution of retrogradely labeled cortical cells in flattened cortex after injections into the superficial and intermediate layers of the SC. Red dots represent retrogradely labeled cells from the FR injections while black dots represent retrogradely labeled cells from the CTB injection site. B: Our reconstruction of the injection sites on a dorsal view of the SC, with red representing the FR injection site and gray representing the CTB injection site. Visuotopic information is based on Lane et al. (1973). C: A photomicrograph of a coronal section of the SC with part of the CTB injection site. The injection site is mainly limited to the superficial layers of the SC. D: A photomicrograph of the coronal section of the superior colliculus with part of the FR injection site with the injection site located within superficial and intermediate layers of the SC. E: A photomicrograph of a myelin section showing the location of MT, MTS, FST, and MTc. F: Close-up view of the location of retrogradely labeled cells with respect to E. Scale bars . 5 mm in A; 0.5 mm in C,D; 2 mm in E,F.

sections crossing cortex into and out of layer 5 two or more times. Most likely there are corticotectal projecting cells between these line formations, but they are probably in adjacent tissue sections processed for myelin, cytochrome oxidase, or in the alternate tracer sections. Unexpectedly, there was a patch of FR labeled cells within dorsal V3 close to V2. This patch was medial and rostral to a patch of CTB labeled cells in V3, but also caudal to another patch of CTB labeled cells in DM. Additionally, there's another patch of CTB labeled cells medial to the FR cells in dorsal V3. CTB labeled cells medial to DM may be within the medial area (area M). Labeled cells were also found within the MT complex, with very few cells within MT itself. FR and CTB labeled cells were found within caudal MST, as well as the dorsal half of FST. Similar to 07-105 (Fig. 4.5), the FR cells were more rostral than the CTB labeled cells within FST. Another cluster, of two patches of both CTB and FR cells, was also present rostral to MTS. Multiple patches of labeled cells were located lateral to FST, again suggesting again the presence of multiple visual areas within IT cortex.

Unlike the cases with injections that are confined to the superficial layers, the deeper injections in this case labeled dense patches of cells within the posterior parietal cortex (PPC) and frontal cortex. Within PPC, the majority of labeled cells were located along the lateral lip of the interparietal sulcus (IPS). These patches spanned the length of the caudal half of the IPS, with branches of cells progressing medially into the IPS. A few more sparse patches of label were found medial to the IPS. In frontal cortex, the borders of FEF had been physiologically defined and lesions were placed along its borders (stars in Fig. 4.6A). Sparse distributions of both CTB and FR cells were present within the locations of the lesion sites, but the majority of labeled cells were located in cortex just

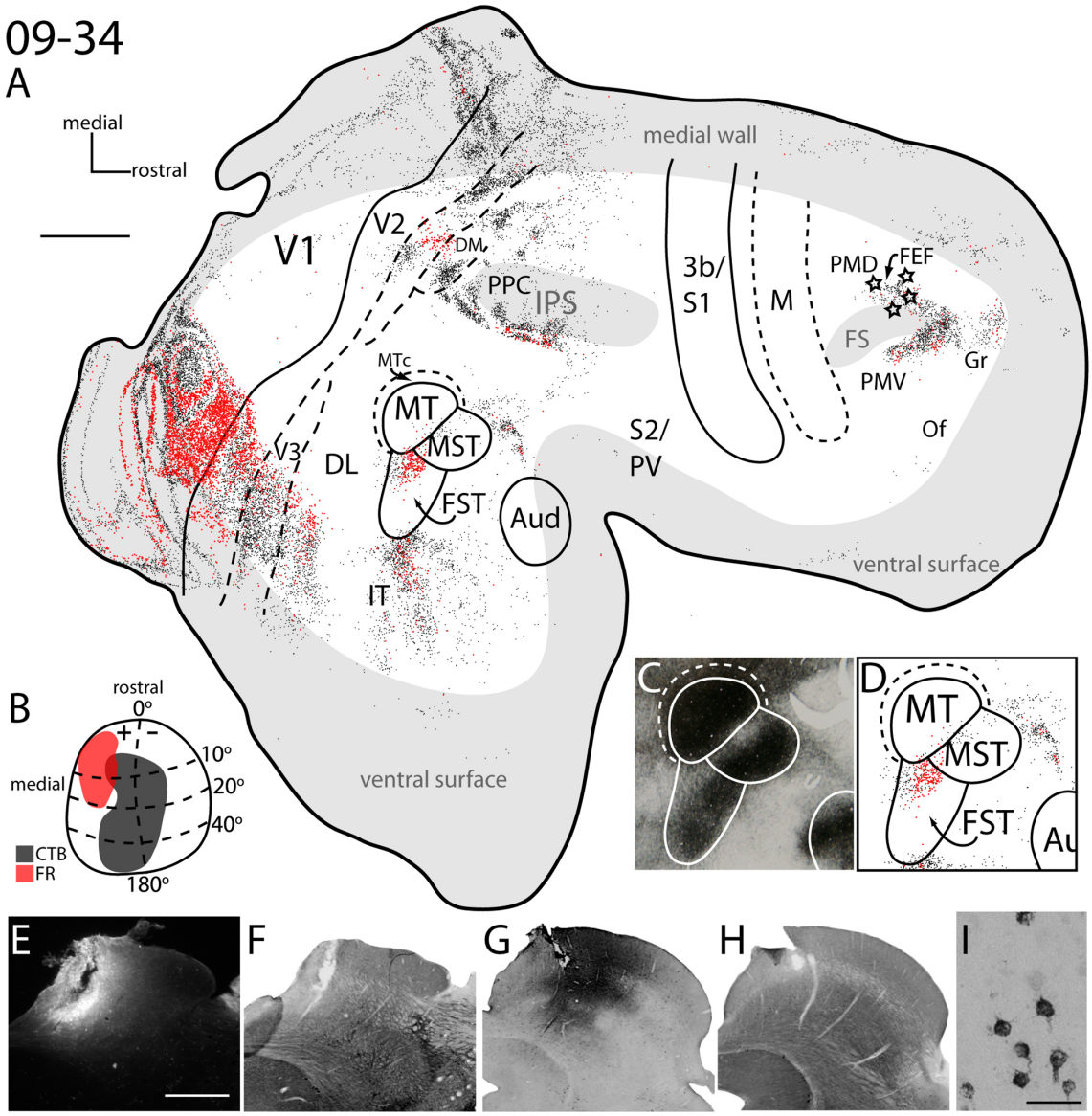

Figure 4.6. Cortical projections to superficial, intermediate, and deep layers of the SC in case 09-34. A: The distribution of CTB (black) and FR (red) retrogradely labeled cells within flattened cortex after injections into the superior colliculus that involved superficial, intermediate, and deep layers of the SC. B: Dorsal view of the SC indicating the locations of the CTB (gray), and FR (red) injection sites. C: A myelin stained section cut parallel to the brain surface showing the locations of MT, MST, FST, and MTc. D: Close-up view of the labeled cells within the MT complex region. E: Photomicrograph of the FR injection site within a coronal brain section through the SC. F: A photomicrograph of an adjacent section to E stained for CO. G: Photomicrograph of the location of the CTB injection site within a coronal view of the SC. H: Photomicrograph of the adjacent CO-stained section to G. I: Photomicrograph of CTB-labeled cells within occipital cortex. Scale bars . 5 mm in A; 2 mm in C,D; 1 mm in E–H; 50 Im in I.

ventral to the FEF (Fig. 4.6A). Multiple patches of label were located ventral and rostral to the frontal sulci extending into granular frontal cortex. Patches of labeled cells were also present within the medial wall, dorsal and rostral to the frontal eye fields. Only a few cells were in the region of S2/PV, and no cells were found within area 3b/S1 or motor cortex.

Case 09-03 had the deepest superior colliculus injection in our study. The CTB injection within the superior colliculus was centered in the SGI, but the tracer spread down to the periaqueductal gray (Fig. 4.7D). As in the other cases, the majority of the injection core encompassed the representation of paracentral vision of the upper visual field. Retrogradely labeled cells were present within the upper field representations of V1, V2, V3, and DL. A dense patch of cells was also present within DM, with and additional dense patch of cells medial to DM, possibly within the medial area. The locations of MT, MST, and FST was estimated based on their expected locations relative to other visual areas and fissures, but it's likely that few, if any cells were present within MT. By location, the majority of cells appear to be within FST. As in previous cases, multiple patches of labeled cells were within IT cortex, as well as rostral to MST, suggesting that multiple areas exist within these regions. A few labeled cells were present within the auditory core (Aud) as well as immediately caudal to the core in the expected region of the auditory belt. Other labeled cells were in the region of S2/PV. Similar to case 09-34 (Fig. 4.6) with injections into deep layers of the SC, dense patches of labeled cells were found just lateral to the IPS, although in case 09-03 the patches of labeled cells extended more rostrally. Patches of labeled cells were distributed rostrocaudally, just lateral to the IPS, with extensions of those patches extending medially toward the IPS. In

frontal cortex, patches of labeled cells along the rostromedial aspect of the frontal sulcus may have included FEF, but the majority of cells were ventral as well as rostral to the expected location of FEF, in prefrontal cortex, and possibly into orbitofrontal cortex. The most caudal of the patches were likely in dorsal premotor cortex. The patches of label and the extents of labeled patches were more dense and expansive than in case 09-34 (Fig. 4.6). Patches of labeled cells were also present in polar frontal cortex of the medial wall and ventral surface. No cells were located within 3b/S1, and only a few were located within all of motor cortex.

In summary, superficial injections produced label in visual cortical areas such as V1, V2, V3, DM, FST, MST, and a small part of IT, with only a few cells present within MT. Deeper injections within the superior colliculus resulted in labeled cells within the posterior parietal cortex, S2/PV, auditory cortex, and frontal cortex with relatively few cells originating from the FEF. No corticotectal projections were observed from primary somatosensory cortex, and only a few from motor cortex in any of our cases.

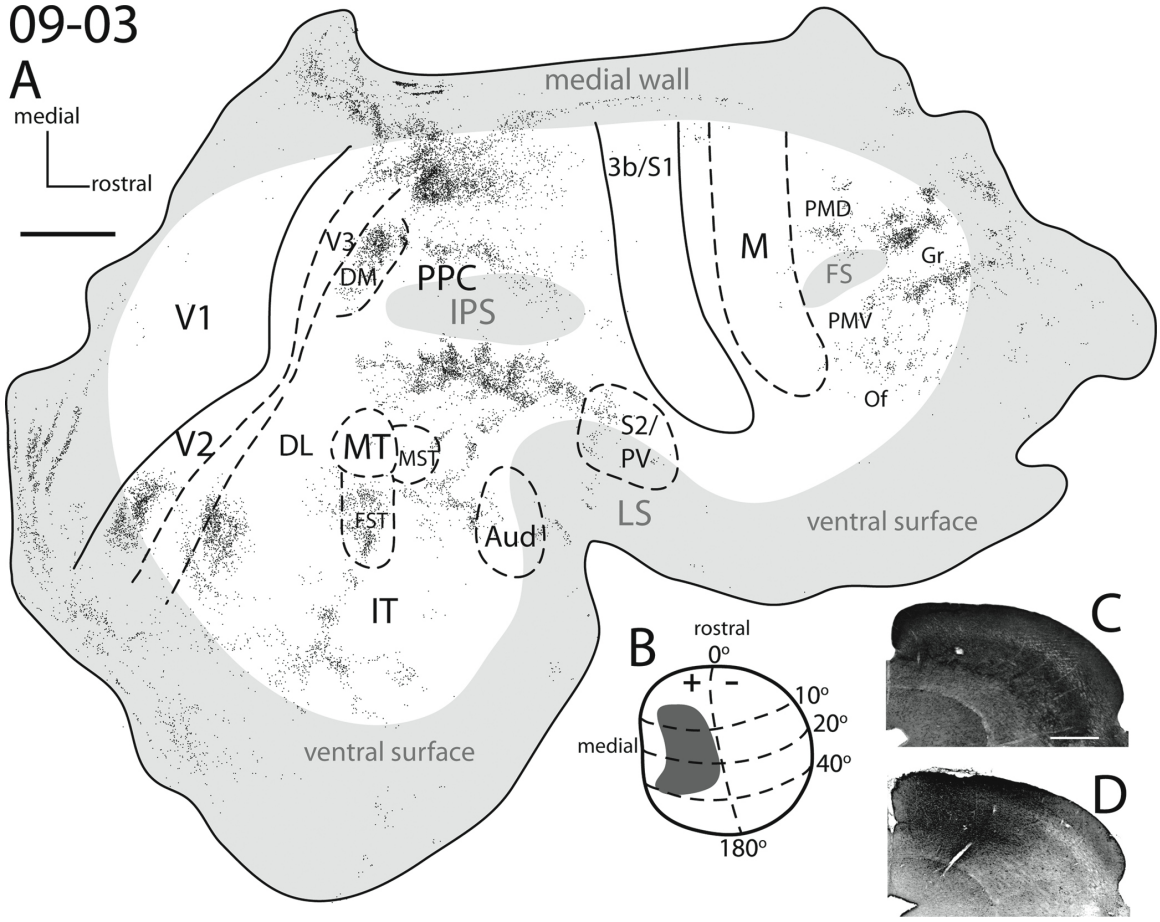

Figure 4.7. Cortical projections to superficial, intermediate, and deep layers of the SC in case 09-03. The distribution of retrogradely labeled CTB cells within flattened cortex after a CTB injection into the deep, intermediate, and superficial layers of the SC. B: Dorsal view of the injection site within the SC. C: Photomicrograph of a coronal AChE section at the location of the injection site core within the SGI. D: Photomicrograph of a coronal section stained for CTB showing the tracer spread through the full layers of the SC. Scale bars . 5 mm in A; 1 mm in C,D.

4.5. DISUCSSION

Prior to this study, most of what was known about corticotectal projections to the superior colliculus in prosimian galagos was obtained from separate studies with injections of tracers into three subdivisions of cortex, MT, DM, and S2/PV (Wall et al. 1982; Beck and Kaas 1998; Wu et al., 2005). In the present study, we determined the distribution of corticotectal projecting neurons from the whole cortex by making injections of retrograde tracers into the superior colliculus. Many of the connections observed in the present study were those expected from observations on other primate species (Fries, 1984, 1985; Cusick, 1988; Lock et al., 2003 Collins et al., 2005), as there were dense projections from early visual areas to the superficial layers of the superior colliculus, while deeper layers of the superior colliculus received projections from visuomotor regions within frontal and parietal cortex. Unexpectedly, few neurons were labeled in MT after any of our superior colliculus injections. Dense projections from MT to the superior colliculus have been reported in New World and Old World monkeys (Graham et al., 1979; Maunsell and Van Essen, 1983; Ungerleider et al., 1984; Cusick, 1988; Lock et al., 2003; Collins et al., 2005;). Instead, we found that in galagos, the majority of corticotectal projections from the MT complex come from FST, and others from MST (Figs. 4.3-7). Another surprising observation was that there were relatively few projections from the FEF to the superior colliculus, although such connections appear to be dense in monkeys (Künzle et al., 1976; Collins et al., 2005; Huerta et al., 1986; Stanton et al., 1988; Komatsu and Suzuki, 1985).

4.5.1. Occipital cortex projections

Our results in prosimian primates are, for the most part, consistent with previous reports in monkeys: that dense, topographically organized inputs to the superficial layers of the superior colliculus originate from early visual areas (Wilson and Toyne, 1970; Tigges and Tigges 1981; Fries, 1984; Lock et al., 2003; Collins et al., 2005). Yet, there were some deviations in our data relative to previous reports. For instance, in macaque monkeys, corticotectal projections from striate cortex to the superior colliculus seem to correlate with cytochrome oxidase staining, with more cells originating from interblob regions than from within blobs (Lia and Olavarria, 1996). In V2, corticotectal projections originate from CO thick stripes (Abel et al., 1997). We did not find a correlation between CO interblobs and the location of labeled cells within striate cortex, and were unable to determine specific connection patterns within V2 because of the lack of CO stripe staining, as reported previously for V2 of galagos (Condo and Casagrande, 1990; Kaskan and Kaas, 2007; Wong and Kaas, 2010). The association between CO modules within V1 and V2, and their connections with the superior colliculus have not been studied in New World monkeys, so it is difficult to say whether connections specific to CO modules reflect a derived trait in all simians or just Old World monkeys.

Retrogradely labeled cells were present within DL (V4) in all studied cases. Most labeled cells were located within the caudal half of DL, much as in New World Monkeys (Cusick, 1988; Collins et al., 2005). Rostral DL is thought to be involved in dorsal stream processing (Weller and Kaas, 1987; Cusick and Kaas, 1988; Kaas and Lyon, 2007).

Although labeled cells were present within DM (V3a) after superficial injections (Fig. 4.3), there were higher densities of labeled cells in this location after deeper

injections (Figs. 4.6 and 4.7). This is consistent with the results of Beck and Kaas (1998) in galagos with DM injections, where terminal label was found within the SO and SGI and even some parts of the SGP. In owl monkeys, DM projects to the lower SGS (Graham et al., 1979).

Just medial to DM, area M (Allman and Kaas, 1976; Krubitzer and Kaas, 1993) contains a map of the complete contralateral visual field with equal amounts of cortex dedicated to central and peripheral vision. In our cases labeled cells were located in this region mainly after deep injections (Fig. 4.4, 4.6 and 4.7). Not much is known about area M, other than it shares connections with V2, DM, areas rostral to MT, and the posterior parietal cortex (Graham et al., 1979; Krubitzer and Kaas, 1993). Area PO of macaques likely corresponds to area M, as PO has connections with posterior parietal cortex, and frontal cortex, as well as occipital visual areas (Colby et al., 1988). Cortex in the PO region has also been called V6 (Shipp et al., 1998).

4.5.2. MT complex projections

Previous reports have suggested strong connections between MT and superior colliculus in New World and Old World monkeys (Collins et al., 2005; Graham et al., 1979; Fries, 1984; Lock et al., 2003) and even connections between MT and the superior colliculus in galagos (Wall et al., 1982). However, the injections by Wall et al (1982) could have involved surrounding areas such as MST, FST, and MTc, particularly since FST appears to project densely to the superior colliculus in galagos. Alternatively, it could be that parts of FST and MST in our present figures are actually part of MT, but that seems unlikely, since most of the label attributed to FST and MST was outside of the architectural borders of MT. Because the lack of evidence for MT projections to the

superior colliculus was surprising, we examined previously published galago cases with MT injections from our laboratory (Wong et al., 2009). Of four cases (07-45, 98-101, 05-40, 06-58 from Wong et al., 2009) with anterograde label in the thalamus and brainstem, we found only one case, which had any detectable terminal label within the superior colliculus. The terminal label was very weak within the SGS of the superior colliculus, but it was in the expected location given the topography of both MT, and the superior colliculus. Given these observations in the present study, and the unpublished data from our previous study, we conclude that the lack of labeled cells within MT after superior colliculus injections reflects a sparseness of projections to the superior colliculus in galagos, and that corticotectal projections from this region of cortex derive mainly from FST, with some from MST. MT also has few connections with FEF in New World monkeys (Weller and Kaas, 1984; Huerta et al., 1987; Krubitzer and Kaas, 1990; Rosa et al, 1993; Tian et al., 1996), and prosimian galagos (Krubitzer and Kaas, 1990; Stepniewska et al., 2009), while studies of connections between MT and FEF in Old World macaque monkeys have produced variable results with some studies reporting sparse or no connections (Maunsel and Van Essen, 1983;Ungerleider and Desimone, 1986), yet others provided evidence for such connections (Andersen et al., 1985; Huerta et al., 1987; Schall et al., 1995). The variability of the connections of MT with FEF and the superior colliculus across primate taxa suggests that MT, an area that may have evolved with primates (Kaas, 2003), has an evolving role in vision, and that an involvement in the production of saccadic eye movements via the superior colliculus and frontal eye fields connections fully emerged in catarrhine primates.

The majority of labeled cells in FST were within the dorsal half, usually close to the MT border. Though we did not differentiate between FSTd and FSTv (Kaas and Morel, 1993), the majority of corticotectal projections were in dorsal FST. FSTd has connections with MT (Kaas and Morel, 1993), and thus MT could indirectly influence the superior colliculus via a relay through FST. In macaques, connections between FST and the superior colliculus have been reported (Lock et al., 2003). In New World monkeys, the corticotectal projections arise from the most dorsal portion of FST (Collins et al., 2005), as in galagos. Labeled cells were also found in MST. This area is thought to be involved in higher order visual motion processing, such as expansion, contraction and rotation, optic flow, object motion, and even smooth pursuit eye movements (Komatsu and Wurtz 1988; Tanaka and Saito 1989; Britten and van Wezel 1998). In galagos, MST also has relatively strong connections with portions of the posterior parietal cortex where defensive movements of the face and forelimb can be evoked by electrical stimulation (Stepniewska et al., 2009). MST in New World and Old World monkeys also has connections with the frontal eye fields (Huerta et al., 1987). We found few cells within MTc/V4t after superior colliculus injections, yet there is some evidence for such connections from V4t of macaques (Lock et al., 2003).

4.5.3. Inferior temporal cortex projections

After injections in the superior colliculus of galagos, multiple patches of retrogradely labeled cells were observed within the inferior temporal (IT) cortex (Figs. 4.3, 4.5, 4.6, and 4.7). While we did not identify divisions within IT cortex in the present cases, architectonic subdivisions of the temporal lobe in galagos have been proposed (Zilles, 1979; Preuss and Goldman-Rakic, 1991a; Wong et al., 2010). The presence of

multiple patches of labeled cells in IT cortex is consistent with the architectonic evidence that several functional divisions exist in this region. The significance of IT projections to the superior colliculus in Old World macaque monkeys is uncertain as one study (Fries 1984) provided evidence for strong connections between IT cortex and the superior colliculus, while another study (Lock et al., 2003) showed rather weak corticotectal projections from this region. Additionally, few projections from IT cortex have been observed in New World monkeys (Collins et al., 2005). Because of the differences in these results, it is difficult to reconstruct the ancestral pattern present in early primates, and more studies are needed.

4.5.4. Posterior parietal cortex projections

In the present study, deep injections in the superior colliculus labeled large population of neurons in a rostrocaudal band of posterior parietal cortex, just lateral to the intraparietal sulcus. The organization of this region of cortex in galagos is not well understood, but the rostral half of this population of labeled neurons lies in a region where electrical stimulation with microelectrodes evokes ear movements, eye lid closure, and face defensive movements (Stepniewska et al., 2005; 2009a). Some of this movement-producing cortex has connections with MST and other visual areas but not MT (Stepniewska et al., 2009b). MT connections appear to be more caudal in posterior parietal cortex (Wall et al., 1982; Kaskan and Kaas, 2007), overlapping the caudal part of the band of superior colliculus projecting cells, where electrical stimulation failed to produce eye or arm movements (Stepniewska et al., 2009a). This unresponsive region in the caudal half of posterior parietal cortex has strong connections to visual areas of occipital cortex (Stepniewska et al., 2009a). The posterior parietal cortex has been

subdivided architectonically by Preuss and Goldman-Rakic (1991a), and homologies with subdivisions of area 7 of macaque have been suggested, but supportive evidence is limited. In macaques, eye movements have been evoked by electrical stimulation of the lateral intraparietal area, LIP, and eye blinking has been evoked from sites just rostral to those that produced saccades (Shibutani et al., 1984; Their and Andersen, 1998). The resulting actions from electrical stimulation of LIP and rostrally adjoining cortex in macaques seem very similar to the eye movements and more rostral eye lid closure zones of posterior parietal cortex in galagos (which are lateral to the intraparietal sulcus), but cortical connections appear to differ. While the movement zones in galagos do not appear to receive inputs from MT and DM (V3a), such inputs to LIP have been reported for macaques (Cavada and Goldman-Rakic, 1989; Blatt et al., 1990; Nakamura et al., 2001); however, Maunsell and Van Essen (1983) used connections with MT to define the ventral intraparietal area VIP, not LIP. The cortex, just lateral to the intraparietal sulcus, in galagos, projects densely to the deep layers of the superior colliculus, as does LIP in macaques (Lynch et al, 1985; Lock et al., 2003; Pare and Wurtz, 1997). Overall, the evidence suggests that a homolog of LIP exists in galagos just lateral to a rostral portion of the intraparietal sulcus. In macaques, and possibly New World monkeys, the region of LIP projects to the frontal eye fields (Huerta et al., 1987; Barbas and Mesulam, 1981; Andersen et al., 1985), but this is uncertain in galagos (Fang et al., 2005). In macaques, face, eye, and arm defensive movements have been attributed to the ventral intraparietal area, VIP (Cooke et al, 2003), and eye, ear, and face defensive movements can be evoked from the rostral part of the zone with dense projections to the superior colliculus in galagos. While the superior colliculus is not usually associated with defensive

movements, such movements have been reported after superior colliculus stimulation in rats (see Schenberg et al., 2005 for review).

After injections of tracer into posterior parietal cortex of New World monkeys, Graham et al., (1979) described projections to the lower layers of the SGS of the superior colliculus. However, Collins et al., 2005 found few labeled neurons in posterior parietal cortex of New World monkeys after injections in the superior colliculus. Our present results, together with those of Graham et al, (1979) suggest that the superior colliculus injections made by Collins et al., (2005) may have been too superficial to label neurons in posterior parietal cortex. Thus, projections from posterior parietal cortex to the superior colliculus are most likely part of a visuomotor network shared by all primates.

4.5.5. Frontal cortex projections

Few, if any, labeled cells were present in frontal cortex after superficial injections into the superior colliculus (Figs. 4.3-5). However, after deep injections, we found cells within frontal cortex, rostral to the caudal half of the frontal sulci (Figs. 4.6 and 4.7). Surprisingly we found few cells within FEF in the case where we used microstimulation to define the boundaries of FEF (Fig. 4.6), and few cells were present in the expected location of FEF, just medial to the rostral end of the frontal sulcus (see Fang et al., 2005 for another galago where the FEF was identified by microstimulation), in the other case with labeled cells in frontal cortex (Fig. 4.7). A lack of FEF projections to the superior colliculus in galagos has also been reported after FEF injections (Stepniewska et al., 2009c). In most other primates where superior colliculus projections have been studied, reports clearly indicate a strong FEF input to the superior colliculus (Künzle et al., 1976; Fries 1984, 1985; Komatsu and Suzuki, 1985; Huerta et al., 1986; Stanton et al., 1988;

Collins et al., 2005). Thus, the lack of evidence for such projections in galagos is surprising. As eye movements can be evoked by electrical stimulation of FEF in galagos, the eye movement may depend on direct projections to brainstem occulomotor neuron pools, rather than by projections to the superior colliculus.

While there were few projections to the superior colliculus from FEF in galagos, there were strong projections within the regions of the dorsal and ventral premotor cortex, and granular frontal cortex. Only a few labeled cells were observed in orbitofrontal cortex after our deepest superior colliculus injections (Fig. 4.7) and these cells could be a result of our injection site spreading into the periaqueductal gray (Leichnetz et al., 1981). When relating the position of our labeled cells in frontal cortex to the motor maps described in Wu et al., 2000, it is likely that the dense patch of labeled cells ventral to FEF along the rostral end of the frontal sulcus could be within a region where ear movements are elicited. The labeled neurons could also be in a region of cortex just ventral to the FEF that has connections with MT in New World monkeys (Krubitzer and Kaas, 1990). Marmosets have cells projecting to the superior colliculus that are located outside of FEF in the ventral and dorsal premotor areas as well as granular cortex (Fig. 2. of Collins et al., 2005). Yet few such cells were identified outside of the FEF in titi and owl monkeys. The lack of cells outside of FEF for these cases is, perhaps, a result of injection sites being limited to the depth of the SGI.

After deep injections into macaque superior colliculus, cells dorsal and anterior to FEF including both areas 6 and 8, as well as cells within the medial wall were labeled (Fries, 1985; Pouget et al., 2009). Goldman and Nauta (1976) described projections from the middle third of the length of the dorsal bank of the principal sulcus to intermediate

and deeper layers of the SC. Leichnetz et al. (1981) suggested that the distribution of prefrontal projections to the superior colliculus was extensive in both New World Cebus and Old World macaque monkeys, with cortex rostral to the arcuate sulcus projecting to the superior colliculus, including cortex of the dorsal bank of the principal sulcus, but not the orbitofrontal cortex. Projections from dorsolateral prefrontal cortex to the superior colliculus may be involved in transmitting information on visuospatial working memory and task-selective signals (Johnston and Everling, 2006, 2009).

4.5.6. Auditory cortex projections

In galagos, only a few labeled cells were found within auditory cortex after deep superior colliculus injections. The location of cells in auditory cortex were likely in both core and belt regions of the auditory cortex. In four cases, labeled cells were present in a dense cluster rostral to MST (Fig. 4.4-7), which coincides with the temporoparietal area (Tpt), a higher order auditory area that has strong connections with frontal cortex (Preuss and Goldman-Rakic, 1991b) and connections with regions of posterior parietal cortex where defensive behaviors are evoked by electrical stimulation (Stepniewska et al., 2009 Figs. 4.3, 4.6, 4.10, and 4.11).

In New World and Old world primates, the deep layers of the superior colliculus contain cells that are responsive to auditory stimuli (Jay and Sparks, 1987; Wallace et al., 1996). The majority of auditory inputs to the superior colliculus are likely from subcortical structures, as only sparse if any corticotectal projections have been reported from primary auditory cortex in New and Old World monkeys (Fries 1984; Collins et al., 2005). However, multisensory cortex between auditory and visual sensory areas may be a source of auditory inputs (Stein et al., 2004).

4.5.7. Somatosensory cortex

In the present cases, no labeled cells were found in primary somatosensory cortex, but some cells were present within the S2/PV region after deep injections into the superior colliculus. This is consistent with previous reports of terminal label within the SGI and SGP layers of the superior colliculus after anatomical tracer injections into S2/PV of galago cortex (Wu et al., 2005), and results from several studies suggesting that primary somatosensory cortex does not project to the superior colliculus (Wu et al., 2005; Collins et al., 2005; Fries, 1984). Projections from PV/S2 to the superior colliculus were sparse in New World monkeys (Collins et al., 2005: Fig. 2), but the injection depths were relatively superficial within the superior colliculus and often did not include lower portions of the SGI or the SGP. Fries (1984) described a weak projection from the region of S2/PV in macaque monkeys, while Lock (2003) did not mention such connections. An orderly somatosensory map is present within the intermediate layers of the superior colliculus (Updyke, 1974; Stein 1976). The majority of somatosensory input to the superior colliculus is thought to arise from subcortical structures such as the cuneate and gracile nuclei along with a few inputs from the spinal cord (Wiberg et al., 1987). However, some somatosensory information reaches the superior colliculus from cortex via S2/PV. Wu et al (2005) suggest that these inputs may not function to guide orienting movements or contribute to the somatotopic representation within the deep layers of the superior colliculus, as inputs from tracer injections localized to a single body structure, such as the face, covered most of the extent of the superior colliculus in galagos. Thus, the projections do not appear to provide somatotopically precise information.

4.5.8. Conclusions

While an optic tectum or superior colliculus is common to all vertebrates, the connections and functions of this structure appear to vary. In all studied mammals, areas of neocortex project to the superior colliculus, where they influence tectal projections to brainstem motor centers and the visual thalamus. Here we provide evidence that early visual areas, V1, V2, and V3, project to the superficial layers of the superior colliculus of galagos as they do in other primates. However, we were surprised by the evidence that two cortical areas that project densely to the superior colliculus in New and Old World monkeys, MT and FEF, do not appear to do so in galagos. The reasons for these differences are unclear. Although, these areas appear to have evolved with primates (Kaas, 2002), they may vary in connections and functions across primate taxa. In cortical regions where cortical areas have been less well defined, differences and similarities across species are less certain. For example, a portion of posterior parietal cortex in both galagos and monkeys project densely to the superior colliculus, but homologues of cortical areas in these two groups remains uncertain. Yet, some features of the projection zone in galagos suggest that this zone contains homologues of LIP and VIP of macaques.

4.6. REFERENCES

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CHAPTER 5

CORTICAL PORJECTIONS TO THE SUPERIOR COLLICULUS IN TREE SHREWS (*TUPAIA* GLIS) [4]

5.1. ABSTRACT

The visuomotor functions of the superior colliculus depend not only on direct inputs from the retina, but also on inputs from neocortex. As mammals vary in the areal organization of neocortex, and in the organization of the number of visual and visuomotor areas, patterns of corticotectal projections vary. Primates in particular have a large number of visual areas projecting to the superior colliculus. As tree shrews are close relatives of primates, and they are also highly visual, we studied the distribution of cortical neurons projecting to the superior colliculus by injecting anatomical tracers into the colliculus. As projections from visuotopically organized visual areas are expected to match the visuotopy of the superior colliculus, injections at different retinotopic locations in the superior colliculus provide information about the locations and organization of topographic areas in extrastriate cortex. Small injections in the superior colliculus labeled neurons in locations within areas 17 (V1) and 18 (V2) that are consistent with the known topography of these areas and the superior colliculus. In addition, the separate locations of clusters of labeled cells in temporal visual cortex provide evidence for five or more topographically organized areas, and evidence that most of temporal cortex has visual

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functions. Injections that included deeper layers of the superior colliculus also labeled neurons in medial frontal cortex, likely in premotor cortex. Only occasional labeled neurons were observed in somatosensory or auditory cortex. Unlike primates, a substantial projection to the superior colliculus from posterior parietal cortex is not a characteristic of tree shrews.

5.2. INTRODUCTION

The superior colliculus is a key structure involved in integrating visual, auditory, and somatosensory information to orienting movements (Schiller et al., 1971, Casagrande et al., 1972; Harting et al., 1973; Stein et al., 1976; Werner et al., 1997; McPeek and Keller, 2004) that are important for navigating environments, avoiding predators, and foraging for food. Differences in how a particular species responds to sensory stimuli to navigate their environment will likely be reflected in the organization of inputs to the superior colliculus. Cortical projections to the superior colliculus have been studied in a wide range of species within the Euarchontoglires clade, which includes primates, lagomorphs, tree shrews and rodents.

In primates, such as New World (Cusick, 1988; Collins et al., 2005) and Old World monkeys (Fries, 1984; Lock et al., 2003), and prosimian galagos (Baldwin and Kaas, 2012), mostly visual and visuomotor areas project to the superior colliculus with primarily visual areas projecting to the superficial layers, and visuomotor areas projecting to deeper layers of the superior colliculus. Few, if any, projections arise from somatosensory areas outside of the region of S2/PV, nor do projections arise from primary motor cortex (Collins et al., 2005; Fries 1984; Baldwin et al., 2012). In contrast, in rodents such as rats and mice, the superior colliculus receives projections from primary somatosensory and motor areas of cortex, as well as from visual areas (Wise and Jones, 1977; Olavarria and Van Sluyters, 1982; Cadusseau and Roger, 1985; Welker et al., 1988; Harvey and Worthington, 1990; Hofsteter and Ehret, 1992; Inoue et al., 1992; Miyashita et al., 1994; Hoffer et al., 2005; Triplett et al., 2009; Aronoff et al., 2010). These nocturnal rodents rely heavily on their whiskers in order to navigate their

immediate environments, while tree shrews, much like primates, navigate their environment visually. Here we consider the cortical projection pattern to the superior colliculus in tree shrews, which are highly visual mammals and members of the Euarchontoglires clade. It is likely that the organization of cortical inputs to the superior colliculus of tree shrews reflects not only features found in other members of the Euarchontoglires clade, but also specializations reflecting their diurnal highly visual niche. Tree shrews have a cone-dominated retina, a large superior colliculus, and a sizeable region of visual cortex that includes large primary and secondary areas as well as an expanded temporal visual cortex (Kaas, 2002; Wong and Kaas, 2009).

The current understanding of cortical projection patterns to the superior colliculus in tree shrews is largely based on the study of Casseday et al. (1979). These investigators divided the cortex of tree shrews into areas based on cytoarchitecture (Fig. 5.1A), as well as descriptions of cortical organization in tree shrews derived from patterns of cortical connections (Diamond et al., 1970; Harting et al., 1973; Casseday et al., 1976; Oliver and Hall 1978). However, our understanding of the cortical organization of tree shrews has changed substantially since the report of Casseday et al., (1979) (Fig. 5.1B). For instance, cortical areas in frontal cortex, including motor and prefrontal cortex, have been further defined using single unit electrode mapping, architecture, and anatomical experiments (Remple et al., 2006, 2007), and our understandings of the location and organization of areas of somatosensory cortex have also been refined and characterized (Sur et al., 1980; 1981; Remple et al., 2006, 2007). Concepts of cortical organization within the visual portions of temporal cortex have been further defined in studies of connections and cortical architecture (Sesma et al., 1984; Lyon et al., 1998; Chomsung et al., 2010), while

the core auditory region of temporal cortex has been defined by microelectrode mapping (Kaas, 2011). Finally, Wong and Kaas (2009) have architectonically analyzed the areal organization of tree shrew cortex using a multitude of histological techniques. The results of all of these studies have produced a substantially different map of the cortical organization (Fig. 5.1B) than the map described by Casseday et al., (1979) (Fig. 5.1A). Therefore, the functional implications of the corticotectal projection patterns in tree shrews need further consideration and reinterpretation.

In the present study, cortical projections to the superior colliculus in tree shrews were studied using anatomical retrograde tracer injections into the superior colliculus. We were able to create injection sites that were small and were located at different topographical locations, as well as at different depths, within the superior colliculus. We analyzed cortical projections to the superior colliculus in flattened preparations of the cortical sheet in order to gain an areal view of the full distribution of cortical projections across all cortical areas. The main goal of this study was to assess the full distribution of cortical projections to the superior colliculus in tree shrews and relate the pattern of projections to known anatomical cortical borders. Additionally, we expected to provide information about the visuotopic organization of temporal and inferotemporal cortical areas by correlating the locations of labeled cells to the topographical locations of injection sites within the superior colliculus as described by Lane et al. (1971). Our results revealed that the primary projections to the superior colliculus in tree shrews arise from visual and visuomotor cortical areas, with few projections from auditory and somatosensory areas. The projections from visual areas 17 and 18 were within topographic locations that largely matched the topographic placement of the injections

within the superior colliculus. Additionally, single injections into the superior colliculus labeled multiple patches of labeled neurons in temporal and inferotemporal cortex that were in register with areal divisions of tree shrew cortex suggested by Wong and Kaas's (2009) architectonic study. Finally, projections from frontal cortex may reflect motor regions that are associated with head or forepaw movements, but are likely outside of primary motor cortex (Remple et al., 2006).

5.3. MATERIALS AND METHODS

Injections of anatomical tracers were placed in the superior colliculus of five tree shrews to reveal the distribution of corticotectal projections. All surgical procedures were approved by the Vanderbilt University Animal Care and Use Committee or the Institutional Animal Care and Use Committee of the University of Louisville and were in accordance with the NIH *Guide for the care and use of laboratory animals*.

5.3.1. Surgical procedures and injections

Surgical procedures have been described elsewhere (Baldwin et al., 2011; Baldwin and Kaas, 2012; Wei et al., 2011). Briefly, tree shrews were initially anesthetized with an intramuscular injection of ketamine (100 mg/kg) and xylazine (6.7 mg/kg), and were maintained at anesthetic levels were maintained during surgical procedures either with isoflurane (0.5-2%) or additional supplements of ketamine and xylazine every 45 minutes. All procedures were performed under aseptic conditions. Once anesthetized, the tree shrews were positioned in a stereotaxic frame. An incision was made along the midline of the skull, and a small craniotomy was made over the occipital lobe and the dura was reflected. After this, one of the following procedures was used to place injections. In the first procedure, the medial wall of the right superior colliculus was visualized after aspiration of the left occipital pole, and retraction of the medial wall of the right hemisphere (Fig. 1C; cases 09-62, 10-18, and 10-20), in the second, the occipital lobe was retracted in order to visualize the caudal aspect of the superior colliculus (Fig. 5.1D, Case 03-34). Once the superior colliculus was visible, injections of 0.2-0.8µl of cholera toxin subunit B (CTB: Molecular Probes Invitrogen,

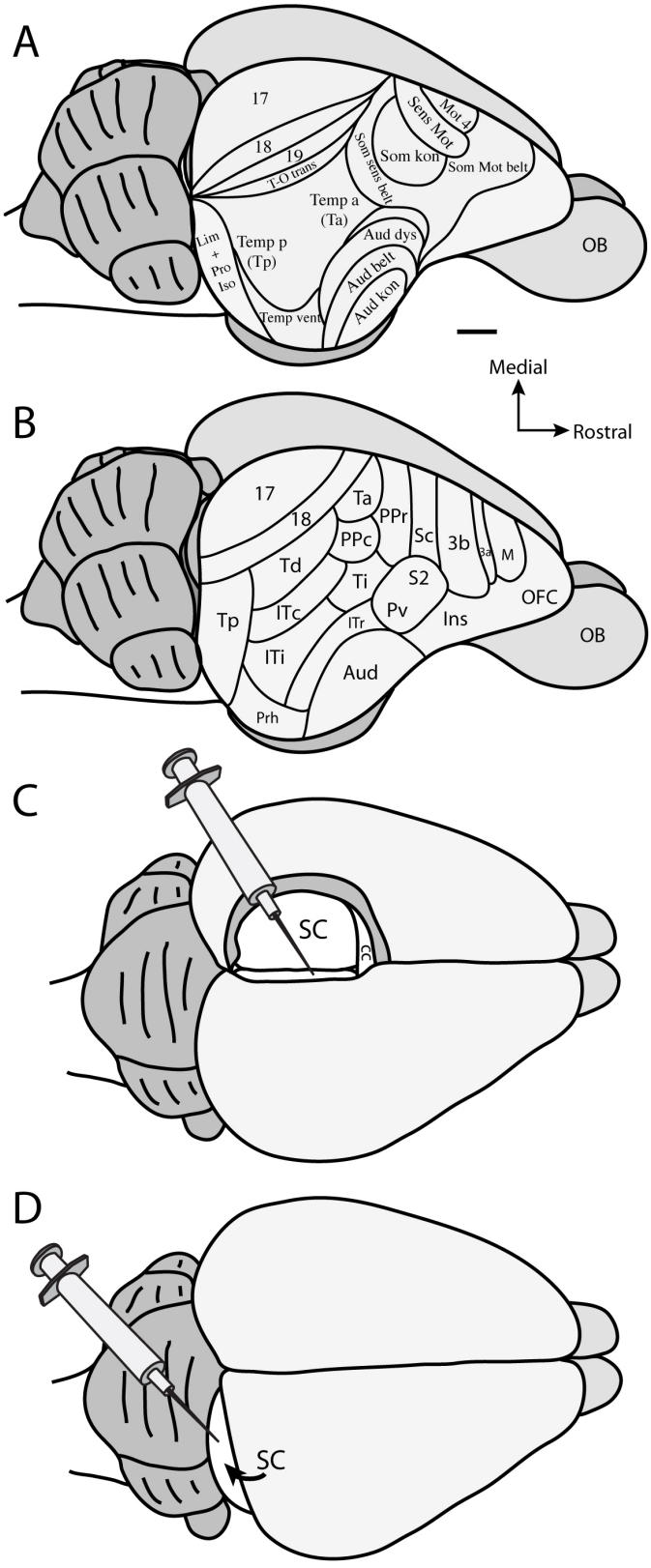
Carlsbad, CA; 10% in distilled water) or fluoro-ruby (FR; Molecular Probes Invitrogen; 10% in distilled

water) were made using a Hamilton syringe fitted to a glass pipette beveled to a fine tip. Finally, in Case 11-35, a glass pipette containing a BDA and CTB mixture (5% BDA and 1% desalted CTB in 0.1M phosphate buffer: tip diameter 2.5 μm) was lowered vertically through cortex, and the tracer was injected iontophoretically (2 μA positive current for 20 minutes) into both the left and right superior colliculus at varying locations. After tracer injections were complete, gelfoam was placed in the region of aspirated cortex, the cortex was covered with gelfilm, and the opening of the skull was sealed with an artificial bone flap made of dental cement. Finally the incision site was closed either with sutures or surgical staples. Tree shrews were then carefully monitored during recovery from anesthesia, and once awake, were given Buprenex (0.03 mg/kg IM) as an analgesic and were returned to their home cage with food and water.

5.3.1. Tissue processing and data analysis

After a 5 to 7 day survival period, the tree shrews were given a lethal injection of sodium pentobarbital (250 mg/kg) and when areflexic, were perfused with phosphate buffer (PB; pH 7.4) followed by 2% paraformaldehyde in PB and 2% paraformaldehyde in PB with 10% sucrose. The brain was then removed, and the cortex was separated from thalamus and brainstem and artificially flattened and placed in 4% paraformaldehyde for 1 hour. The brainstem and thalamus were also placed in 4% paraformaldehyde for 1 to 2 hours. After postfixation, brain tissue was placed in PB with 30% sucrose for twelve to twenty four hours at 4 ^{o}C for cryoprotection.

Figure 5.1. *Organization scheme of tree shrew cortex based on* ***A.*** *Casseday et al., 1979, and* ***B.*** *adapted from Wong and Kaas, 2009.* ***C.*** *Illustration of anatomical tracer placement after aspiration of the contralateral hemisphere and retraction of the ipsilateral hemisphere to the injected superior colliculus.* ***D.*** *Illustration of anatomical tracer placement after retraction of the occipital lobe to visualize the caudal aspect of the superior colliculus. See abbreviations section in current report for list of abbreviations. Scale bar is 2mm.*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The cortex was cut parallel to the pia surface, and the brainstem and thalamus were cut coronally at a thickness of 40 μm on a freezing microtome. Cortical sections were divided into three or four series of every third or fourth section. One series was mounted directly onto glass slides without further processing for the visualization of neurons labeled with the FR tracer. Another series was processed for CTB using an immunohistochemical protocol (Baldwin et al., 2011). The third and fourth series were processed for cytochrome oxidase (Wong-Riley, 1979) or myelin (Gallyas, 1979). The brainstem and thalamus sections were saved in series of five with one series mounted directly onto glass slides for FR injection site analysis; one series processed to reveal CTB injection sites; and a third processed for CO; while the fourth and fifth series were processed for acetylcholinesterase (AChE: Geneser-Jensen and Blackstad, 1971), Nissl, or saved for another study.

Locations of retrogradely labeled cell bodies were plotted using a Neurolucida system (MicroBrightField, Williston, VT). Cortical tissue overlying the superior colliculus injection sites was analyzed for possible tracer contamination within our processed tissue and during dissection. Photomicrographs of tissue sections were taken using a DMX1200F digital camera mounted to a Nikon microscope (Nikon Inc., Melville, NY) or were taken with Qimaging EXi Aqua digitizal camera (Surrey, BC, Canada) mounted to a Leica microscope. Photographs were adjusted for brightness and contrast using Adobe Photoshop but were otherwise unaltered. The locations of injection sites and retrogradely labeled cells in sections processed for CTB or FR were aligned with sections processed for architectonic features using common blood vessels. Injection site locations relative to superior colliculus layers were determined by alignment with

sections processed for CO, Nissl, or AChE, while borders of cortical areas were determined using CO- or myelin-stained sections.

5.3.2. Injection site identification

The superior colliculus of tree shrews can be divided into seven main layers (Fig. 5.2), and these layers can be identified using CO and AChE staining. These layers include the stratum zonale (SZ), the stratum griseum superficial (SGS), and the stratum opticum (SO), stratum griseum intermediale (SGI), stratum album intermediale (SAI), stratum griseum profundum (SGP), and the stratum album profundum (SAP) (Fig. 5.2). In CO-stained sections, SGI and SGS, and SAP can be identified by their dark CO staining, while the other interleaving layers stain moderately or lightly for CO. Within the SGS, two sublayers can be identified based on Nissl (Abplanalp, 1971) and CO staining with the upper layers staining more darkly for CO than the lower layer (Lee and Hall, 1995) (Fig. 5.2A). The SO has a heterogeneous appearance with fibers staining darkly for CO and with non-CO staining tissue between the fibers. Like other species (Wiener, 1986; Bickford and Hall, 1989; May and Porter, 1992; Baldwin et al., 2011; Balaram et al., 2011), the SGI in tree shrews can be divided into sublayers, but we do not define these subdivisions in the present report. SGP stains slightly darker for CO than SAI but each layer contains fibers predominately moving in a medial-lateral direction. SAP also stains lightly for CO. In AChE preparations, the SGS stains darkly, but most other layers contain darkly staining AChE fibers passing through non-staining tissue. The difference in the direction of the fiber paths is much more apparent in AChE sections, yet determining the borders between the SAI, SGP, and SAP is difficult. Additionally, there may be a slight difference in the staining/fiber density of the medial SGI compared with

the lateral SGI (Fig. 5.2B), which could simply reflect the abrupt curvature along the medial wall of the superior colliculus. The superficial layers, including the SZ, SGS, and SO are primarily associated with visual sensory functions; while the deeper layers are associated with higher order visual functions, the integration of sensorimotor inputs, as well as various motor functions (Casagrande et al., 1972; Harting et al., 1973; Casagrande and Diamond, 1975; Raczkowski et al., 1976; Albano et al., 1978). Often our injections involved multiple layers; however, we were still able to compare cases with mainly superficial injections that included the upper half of the SGI (Cases 09-62, 03-34, 11-35RH), and cases with injection sites that included deeper, or all, layers of the superior colliculus (cases 11-35LH, 10-18, 10-20).

The visuotopic organization of the superior colliculus in tree shrews has been determined in microelectrode recording experiments (Lane et al., 1971). The medial superior colliculus contains cells responsive to stimuli within the upper visual field, and the lateral superior colliculus contains cells responsive to stimuli within the lower visual field. The caudal aspect of the superior colliculus represents the peripheral visual field, with central vision represented more rostrally. We did not individually determine the visuotopic location of the injection sites but instead based these locations on the maps of Lane et al. (1971) after reconstructing the injection site locations within a dorsal view of the superior colliculus.

For all but two injected hemispheres, case 11-35L and 11-35R, we found no evidence of cortical contamination. However, these cases did show slight contamination artifact within area 17. Previous reports on cortical connections in tree shrews suggest

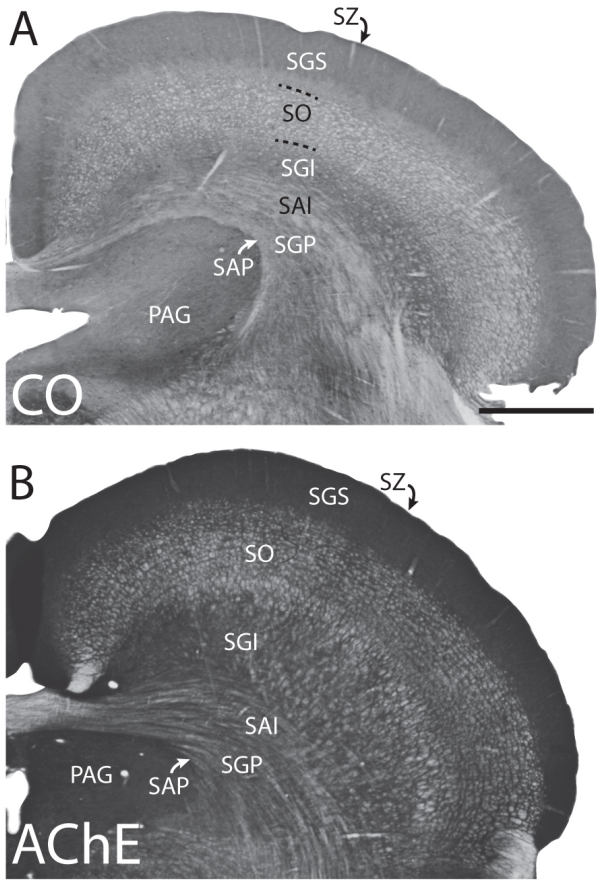


Figure 5.2. *Laminar organization of the superior colliculus as revealed through cytochrome oxidase (CO) staining (top section) and acetylcholinesterase (AChE) staining (bottom section). The seven main layers of the superior colliculus are the stratum zonale (SZ), the stratum grisium superficiale (SGS), the stratum opticum (SO), the stratum griseum intermediate (SGI), the stratum album intermedium (SGI), the stratum griseum profundum (SGP), and the stratum album profundum (SAP). Also shown is the periaqueductal grey (PAG). Medial is left, and dorsal is up. Scale bar is 1mm.*

that only areas 18, Td, and Tp share connections with area 17 (Sesma et al., 1984; Lyon et al., 1998) and therefore we still present cases 11-35R and 11-35L. For case 11-35R, the contamination of area 17 was minor; however, 11-35L may have significant contamination of area 17, as suggested by two distinct foci of label within areas 18, Tp, and Td. This case still provides useful information on the organization of temporal visual areas that do not receive projections from area 17 (Sesma et al., 1984; Lyon et al., 1998).

5.3.3. Determining the locations of labeled cells

Most cortical cells projecting to the superior colliculus in tree shrews arise from layer 5 (Casseday et al., 1979). Though it is difficult to locate the laminar position of labeled cells when cortex is cut parallel to the pia surface, few, if any, labeled cells were present within our most superficial sections of cortex, and, instead, were present predominantly within the bottom half of our samples, likely below layer 4.

We identified cortical areas in the flattened cortex of tree shrews using tissue sections processed for CO or myelin (Fig. 5.3), or by relating the position of labeled cells to cortical maps described by Wong and Kaas (2009) (Fig. 5.1B). Areas 17, 3b, S2/PV, auditory cortex (Aud), as well as the orbitofrontal cortex (OFC) were identified by their characteristic dark CO and myelin staining patterns (Wong and Kaas, 2009) (Fig. 5.3). Area 18 stains less darkly for myelin than area 17, but more darkly than rostral and lateral cortical areas. Determining the boundaries for subdivisions of temporal cortex (Tp, Td, Ta, Ti, and IT) was more difficult using CO and myelin. Thus, we estimated the locations of these borders as determined in Wong and Kaas (2009), who used additional histological staining techniques to determine border locations. To be conservative, we avoided placing most of these borders in our illustrations, and only indicated the expected locations of areas.

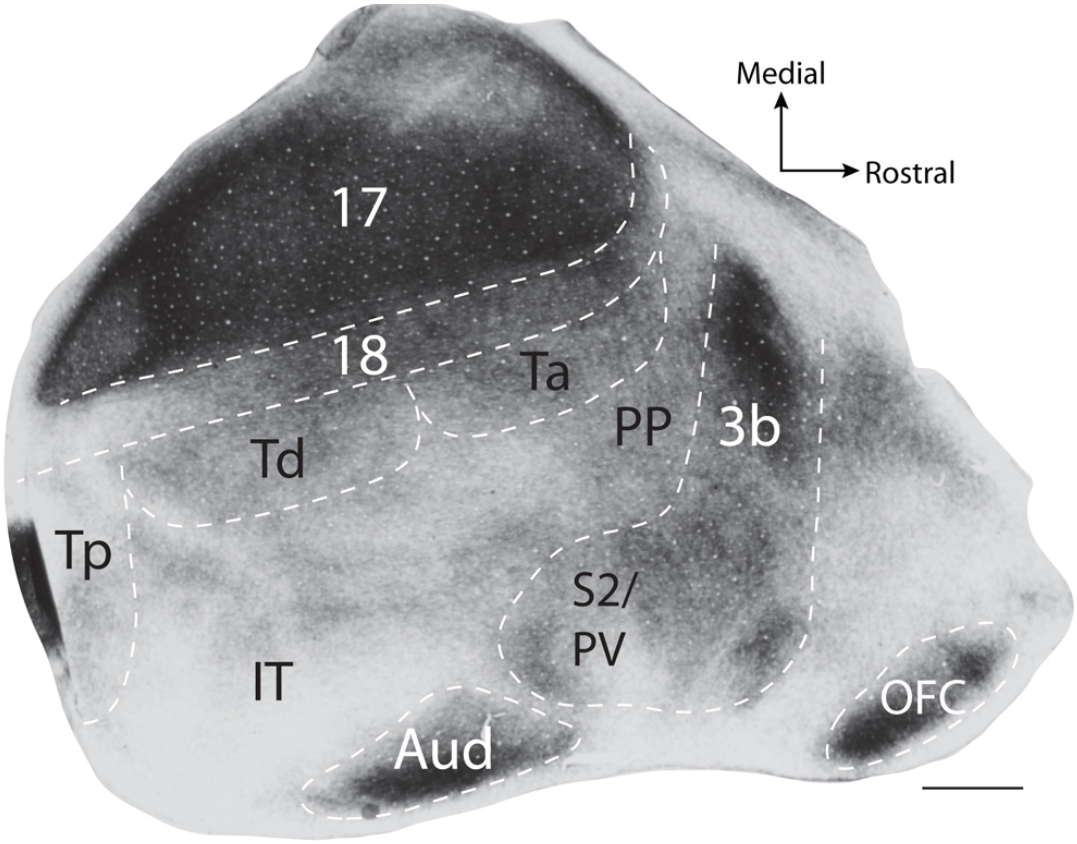


Figure 5.3. *Cortical architecture revealed by myelin staining. Area 17, auditory cortex (Aud), area 3b, and the orbital frontal cortex (OFC) stain darkly for myelin, while PV/S2 and area 18 stain slightly less darkly but more so than surrounding cortical tissue. The dark myelination caudal to Tp is likely a result of uneven folding within this region of cortex. Scale bar is 2mm.*

5.4. RESULTS

Patterns of corticotectal projections in tree shrews were revealed by placing injections of tracers in the superior colliculus. For this study, ten anatomical tracer injections were placed into six superior colliculi of five tree shrews. Of these cases, five injections involved superficial and intermediate layers of the superior colliculus, while five injections involved superficial, intermediate and deep layers of the superior colliculus. The depths of injections were determined by aligning coronal sections processed for tracers with adjacent anatomical sections processed for CO, or AChE. Results from the superficial injection cases are presented first. We expected visual areas to project most superficially in the superior colliculus and frontal visuomotor areas to project to deeper superior colliculus layers.

5.4.1. Cortical projections

Case (09-62: Fig. 5.4), contained the most superficial injections. The injection sites were located within the lower SGS, SO, and dorsal most aspect of the SGI. Both CTB and FR injections were in close proximity to one another along the medial wall of the superior colliculus (Fig. 5.4), a location that represents paracentral vision of the upper visual field (Lane et al., 1971). As the injection cores were small in this case, limited numbers of cells were labeled in cortex. Labeled cells in cortex were present within areas 17, 18, Tp, as well as a few patches of cells located within the IT region. Cells within area 17 and 18 were in locations of upper visual field representations close to the border between 17 and 18 representing the vertical meridian (Kaas et al., 1972), consistent with the retinotopic locations of the injection sites. Area 18 contained two patches of labeled cells, which may reflect the presence of modular subdivisions within area 18 (Sesma et

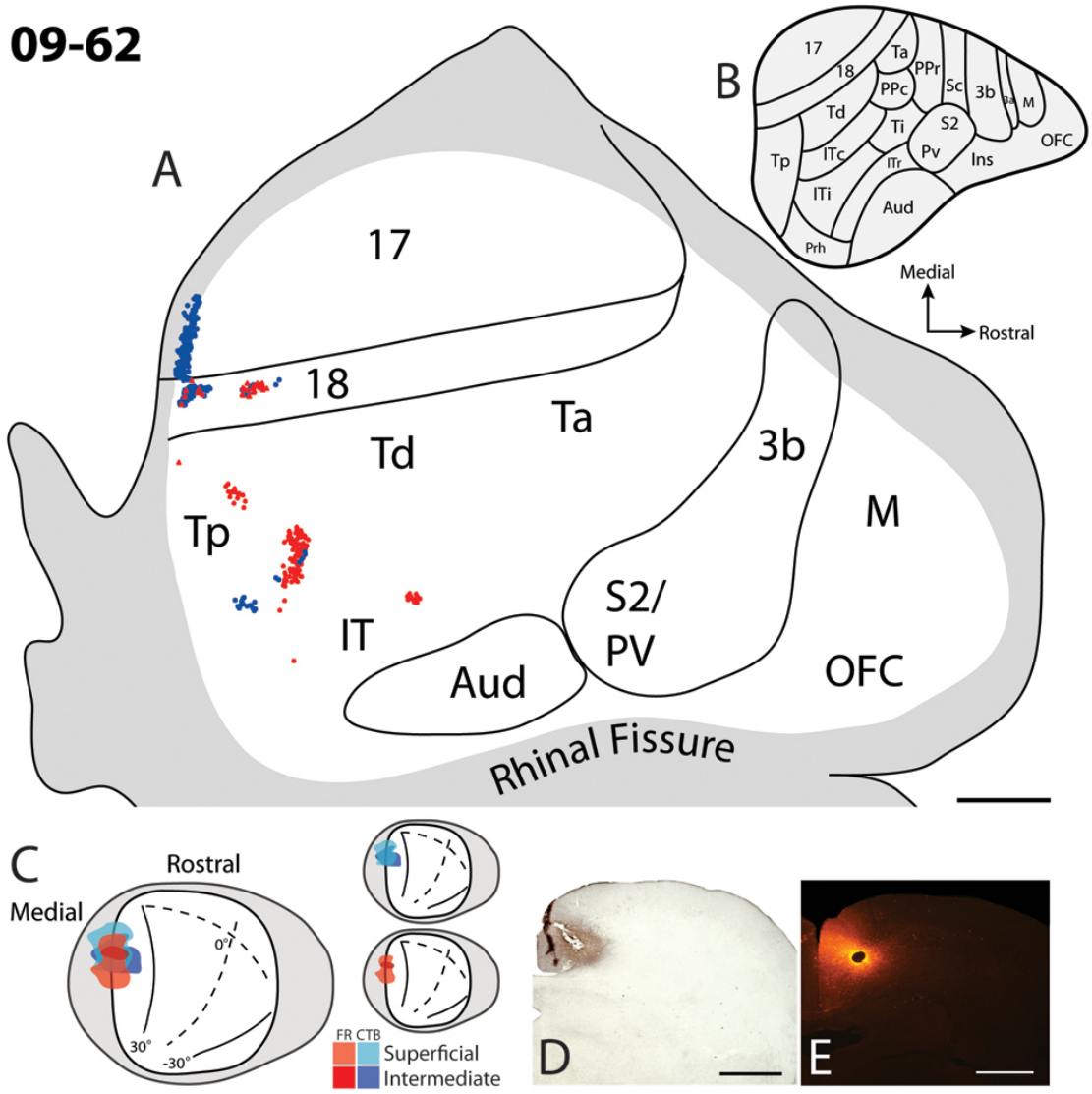


Figure 5.4. *Cortical projections to the superficial and intermediate layers of the superior colliculus in case 09-62.* ***A****. The distribution of retrogradely labeled cells within the flattened cortex after fluoro-ruby (FR) and cholera toxin subunit B (CTB) injections into the superior colliculus. Solid lines represent borders determined using myelin stained sections, and the grey shaded region represents cortex that was either along the medial or ventral surfaces of the brain. Red dots represent the locations of retrogradely labeled FR cell bodies, while blue dots represent the locations of retrogradely labeled CTB cell bodies.* ***B****. Is a dorsal view reconstruction of the location of injection sites. Red hues represent the location of the FR injection site, while blue hues represent the location of the CTB injection site. Darker hues indicate the location of injection sites deeper within the superior colliculus.* ***C****. Photomicrographs of the CTB (left) and FR (right) injection sites in coronal sections through the superior colliculus. Scale bar for A is 2mm, and D and E is 1mm.*

al., 1984; Lyon et al., 1998). Few labeled FR cells were found in area 17, and this is likely because the injection site was centered within the SO of the superior colliculus and did not include much of the upper SGS, which is known to receive striate projections (Harting and Noback, 1971; Casseday et al., 1979; Huerta et al., 1985). No labeled cells were present within motor, somatosensory, or auditory cortical areas. Thus, injections including the SGS and SO labeled cells within early visual areas such as 17 and 18, as well as some temporal visual areas.

The second case, 03-34 (Fig. 5.5), contained injection sites that were slightly deeper within the superior colliculus. Both CTB and FR injections were within the most caudal aspect of the superior colliculus representing peripheral vision. The injection sites included superficial layers of the superior colliculus as well as the SGI. The location of the FR injection site was within the upper visual field within the representation of peripheral vision, while the CTB injection site was near the representation of the horizontal meridian including both upper and lower visual fields. The FR injection was more medial than the CTB injection in the superior colliculus, and the two injection sites did not overlap. Labeled cells were located within area 17, 18, Tp, Td, Ta, Ti and possibly the posterior parietal cortex; multiple patches were also located within IT and the outer most edge of auditory cortex. No cells were located within area 3b, S2/PV, or OFC. Additionally, a few scattered FR cells were located along the rostral medial wall of frontal cortex, possibly including premotor cortex. Within cortical areas, patches of labeled cells for each of the two injections were displaced from one another and rarely overlapped, suggesting the locations of borders and a retinotopic organization pattern throughout temporal and occipital cortex. Most importantly, a band of cortex between Td

and auditory cortex has repeating patches of neurons labeled by the two injections, suggesting that peripheral vision is represented back to back in the ITi/Ti bordering regions (compare label in Fig 5.5A with map in 5.5B). The appearance of multiple patches of alternating repeating patterns of reversed label within this region suggests a modular organization within these areas and a complex visuotopic organization. A similar array of alternating patches of label for the two injections extended mediolaterally along the presumptive border of Td and Tp. Patches of labeled neurons representing peripheral vision were also located along the caudal border of Tp and rostrally between Ti and PPc. Only one patch of labeled cells for each tracer was apparent within Tp. A band of cells was located ventral to Td, possibly within ITc. Finally, patches of labeled neurons representing peripheral vision were located in the ventral part of area 17 that was unfolded in the flattened cortex. This part of area 17 is known to represent peripheral vision (Kaas et al., 1972). Other labeled neurons were in rostral and caudal area 18 locations, also representing peripheral vision. The scattering of labeled cells in lateral IT suggests a lack of visuotopy in this region.

The next set of superior colliculus injections (11-35R and 11-35L, Figs. 5.6 and 5.7) were iontophoretically placed in the superior colliculi of the left and right hemispheres of the same tree shrew. These two injections involved the lateral half of the superior colliculus within the intermediate and deeper tectal layers. Since it was not possible to visualize the lateral half of the superior colliculus by ablating part of the opposite cerebral hemisphere, injections were placed by penetrating the overlying visual cortex with a glass pipette in order reach the lateral superior colliculus. While injections

were successfully placed in the superior colliculus of each midbrain using this procedure, a slight

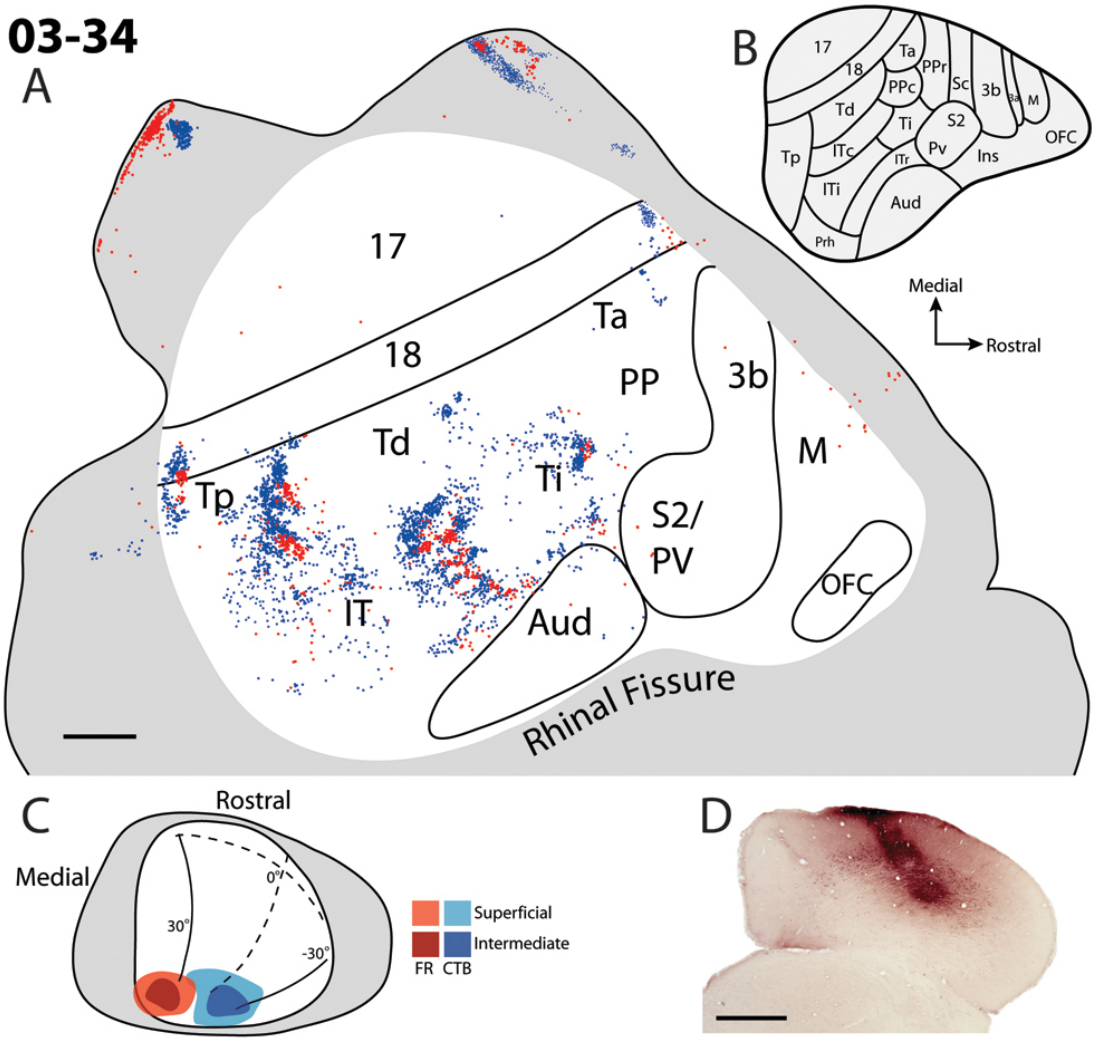


Figure 5.5. *Cortical projections to the superficial and intermediate layers of the superior colliculus in case 03-34.* ***A****. Reconstruction of the labeled cells within flattened cortex. Blue dots represent the location of retrogradely labeled CTB cell bodies, while red dots represent the location of retrogradely labeled fluoro-ruby cell bodies.* ***B****. Shows the location of architectonically defined borders within the tree shrew cortex as determined by Wong and Kaas, 2009.* ***C****. Dorsal view reconstruction of the injection site locations within the superior colliculus.* ***D****. Photomicrograph of the CTB injection site within a coronal section through the caudal aspect of the superior colliculus. Scale bar A is 2mm, and D is 1mm.*

contamination of parts of striate cortex along the course of the pipette penetration occurred in each attempt. Results are included here because the amount of labeled transport to neurons elsewhere in cortex appeared to be quite small, and because the labeled neurons outside of area 17 would only be in locations known to project to area 17 (areas 18, Tp, and Td: Sesma et al., 1984; Lyon et al., 1998) and not other areas projecting to the superior colliculus. Thus, the injections provide useful additional information.

Of the two injections in the superior colliculus in case 11-35, the more lateral and superficial injection was in the right superior colliculus, 11-35R (Fig. 5.6). The injection core was focused within the SO and SGI, while avoiding the SGS. As a result, very little label was in area 17 and 18, which project to the SGS; and the labeled neurons in these areas may reflect some involvement of the SGS, or in part, the slight contamination of area 17. However, the dense patches of labeled neurons in cortex lateral to area 18 completely, or nearly completely, reflect the injection in a lateral part of the superior colliculus that represent paracentral vision of the upper visual quadrant. Two patches of labeled cells were observed within area Tp with one patch seemingly denser than the more caudal patch of cells. An additional patch of labeled cells was present lateroventral to these two patches, and was possibly also within Tp. Similar to previous cases a patch of labeled cells was observed within Td, as well as the Ti-Tp region. The scattering of labeled neurons in IT cortex provides further evidence that this cortex is likely visual but without much visuotopic organization. While a few labeled neurons were in auditory cortex, none were in somatosensory cortex or posterior parietal cortex. Additionally, as in

previous cases with injections that did not penetrate beyond the SGI, no labeled cells were observed in frontal cortex.

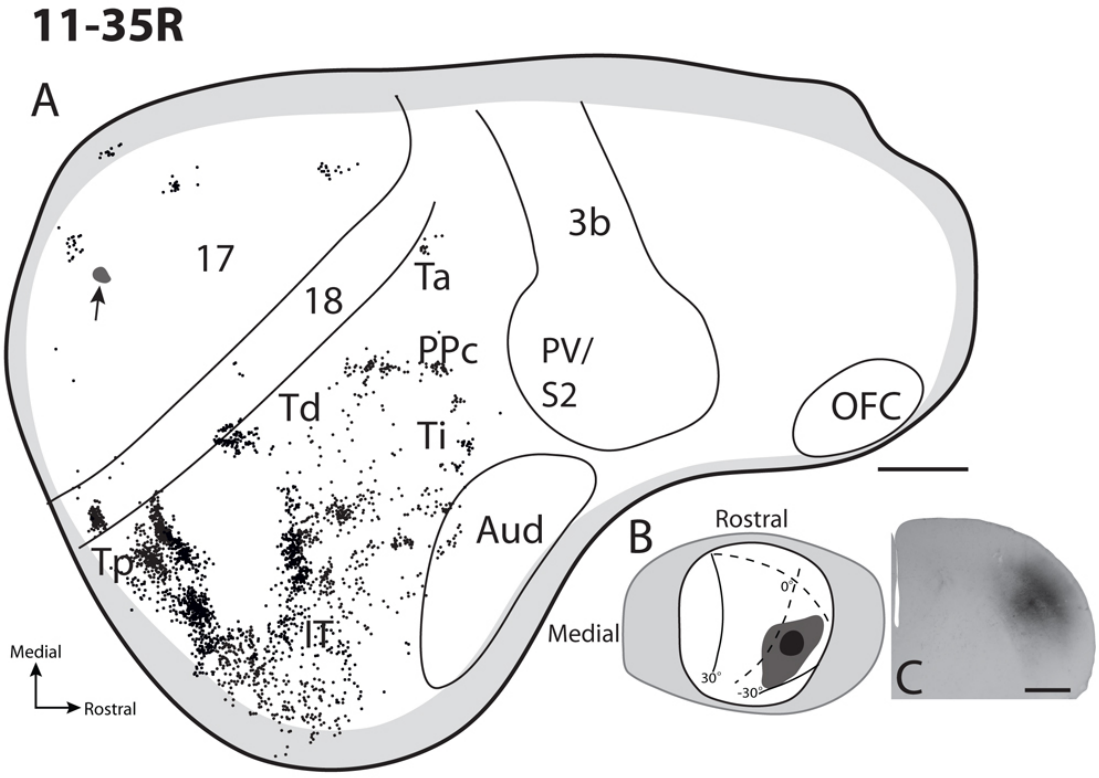


Figure 5.6. *Cortical projections of the superficial and intermediate layers of the superior colliculus in case 11-35R.* ***A****. The distribution of retrogradely labeled CTB cells throughout cortex after an injection into the central lateral superior colliculus. The small black shaded region in area 17 with an arrow shows the location of the penetration track during tracer placement.* ***B****. Dorsal view reconstruction of the injection site superimposed with a visuotopic map described by Lane et al., 1971.* ***C****. Shows a photomicrograph of a coronal section through the superior colliculus indicating the depth of the CTB injection site. Scale bar for A is 2mm, C is 1mm.*

The injection in the left superior colliculus of case 11-35L (Fig. 5.7), involved the middle of the superior colliculus representing paracentral to peripheral vision close to the horizontal meridian. The injection core included the lower SGS, SO, and much of the SGI. Labeled neurons were observed in the expected topographic location of area 17; while labeled cells within the rostral aspect of area 18 correspond to paracentral vision of

the lower visual quadrant near the horizontal meridian, roughly matching the injection site in the superior colliculus. While some of the patches of

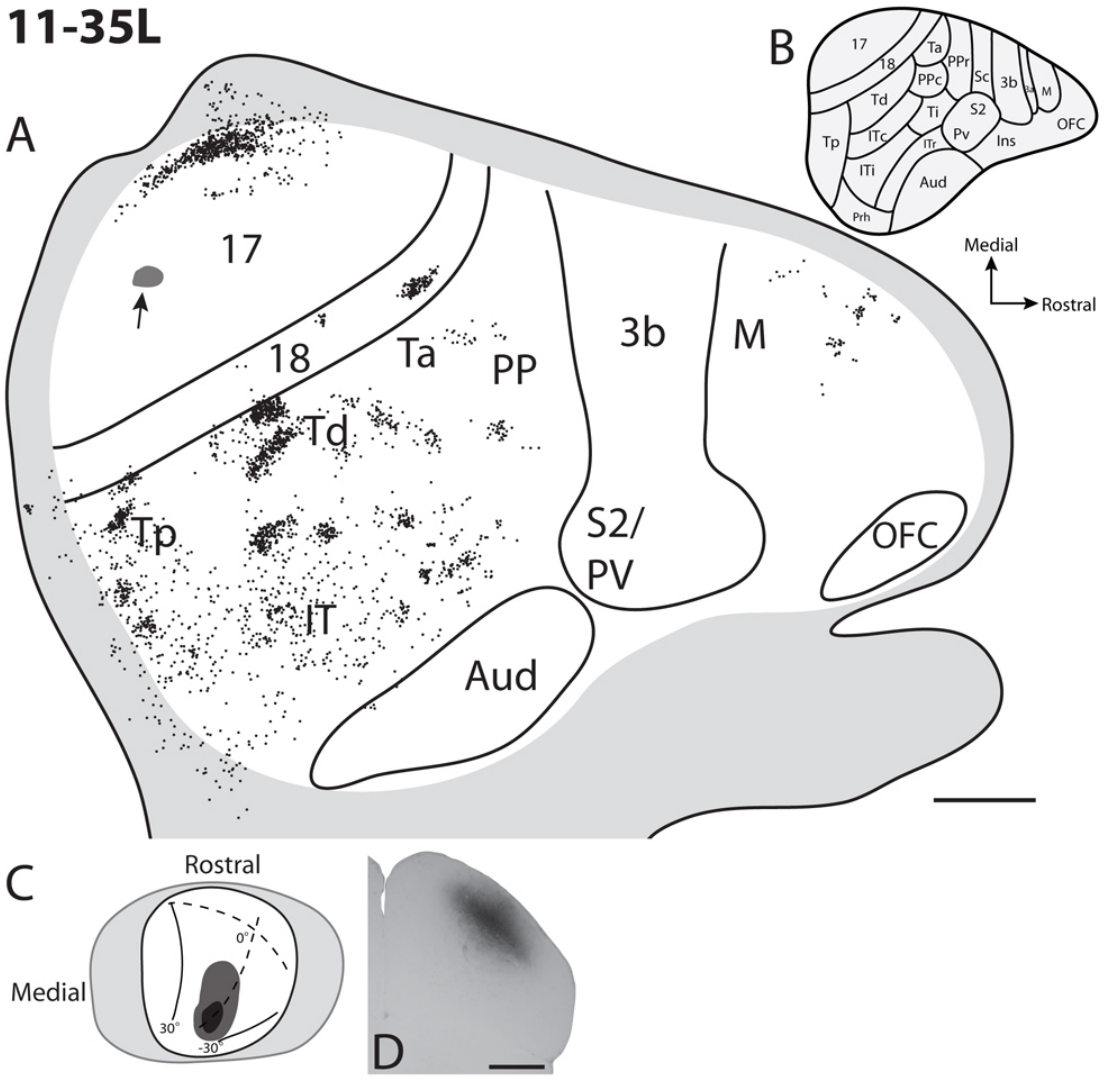


Figure 5.7*. Cortical projections to the superficial, intermediate, and deep layers of the superior colliculus in case 11-35L.* ***A****. Reconstruction of the distribution of labeled cells within flattened cortex of the left hemisphere. The cortex has been flipped to ease comparisons of cortical label with other cases. The small black shaded area with arrow in area 17 represents the location of the penetration track during tracer placement.* ***B****. Cortical organization map adapted from Wong and Kaas 2009.* ***C****. Dorsal view reconstruction of the superior colliculus superimposed with the visuotopic map by Lane et al., 1971. In this case the darker grey represents the core of the injection site, while the light grey represents the tracer spread.* ***D****. Photomicrograph of a portion of the CTB injection site in a coronal view of the superior colliculus. Scale bar for A is 2mm, for D 1mm.*

labeled neurons in Tp and Td could reflect the slight contamination of area 17, the bulk of the label in temporal, parietal, and frontal cortex likely reflect projections to the superior colliculus. Patches of label in this case provide further evidence for retinotopic areas in the upper region of temporal cortex, a lack of retinotopy in ventral IT, and a projection from dorsomedial frontal cortex to the deeper layers of the superior colliculus.

The CTB injection core for case 10-18 (Fig. 5.8) extended into the deepest layers of the superior colliculus. This long injection core was obtained by placing two injections at different depths within the superior colliculus along a diagonal trajectory arising from the medial wall. These separate injections were positioned within the upper SGI as well as the deep layers of the superior colliculus, and there was a slight gap in the spread of the injection core within the lower SGS (Fig. 5.8E). Additionally, some tracer spread into the dorsal-most aspect of the central grey (Fig. 5.8E). Labeled cells were found in area 17, 18, Tp, Td, IT, as well as within the frontal cortex. The foci of labeled cells were in the upper visual field representations within area 17 and 18 and were close to the border between area 17 and 18 representing the vertical meridian, consistent with the injection site location near the medial margin of the superior colliculus within the superficial layers. Similar to cases, 09-62 (Fig. 5.4) and 10-20 (Fig. 5.9), multiple patches of labeled cells were present within area 18, suggesting a modular organization. Also, as in case 10-20, two patches of labeled cells were present within the region of Tp, with one more focused patch located rostromedial to a more diffuse patch. The rostromedial patch is further away from the rostral area 18 border than the more diffuse patch as in the previous case 10-20 (Fig. 5.9). Cells labeled within the Td region were located close to the rostral border of area 18. Again, a band of cells was located ventral to the Td region

but rostral to the Tp region within IT, much as in case 10-20 (Fig. 5.9); however, the gap between the cell clusters was much larger for case 10-18. Labeled cells within the ventral IT region were scattered, suggesting a lack of a retinotopic organization. Foci of retrogradely labeled cells within frontal cortex were in locations similar to those observed in case 10-20 (Fig. 5.9), with one patch located caudolaterally to the more rostromedial patch. These cells were likely within a premotor area rostromedial to the primary motor areas (Remple et al., 2006, 2007). Some labeled cells within the caudal patch could be within the most rostral aspect of primary motor cortex. Surprisingly, no cells were located within the Ta/Ti/PPc region. Again, no cells were present within somatosensory areas 3b, S2/PV, orbital frontal cortex; and only a few cells were within the architectonically defined auditory cortex. Overall, the results observed in case 10-18 were similar to case 10-20 with multiple patches of labeled cells within the region of Tp and area 18; labeled cells within IT and frontal cortex; and a lack of labeled cells within somatosensory, primary motor, and auditory cortex.

The injection cores for our final case (10-20 Fig. 5.9) extended into the deepest layers of the superior colliculus (Fig. 5.9 D-F) and were in similar topographic locations as the injection sites for cases 09-62 (Fig. 5.4) and 10-18 (Fig. 5.8). The full extent of the CTB injection core covered all layers of the superior colliculus. This extent was achieved by placing tracer injections at two different depths along the injection tract. One focus was within the superficial layers of the superior colliculus (Fig. 5.9D), while the other was within the SGI (Fig. 5.9F). The two foci for the FR injection were within the lower SO/upper SGI, and the deep layers of the superior colliculus (Fig. 5.9E). Both CTB and FR injections were positioned diagonally into the superior colliculus from the medial

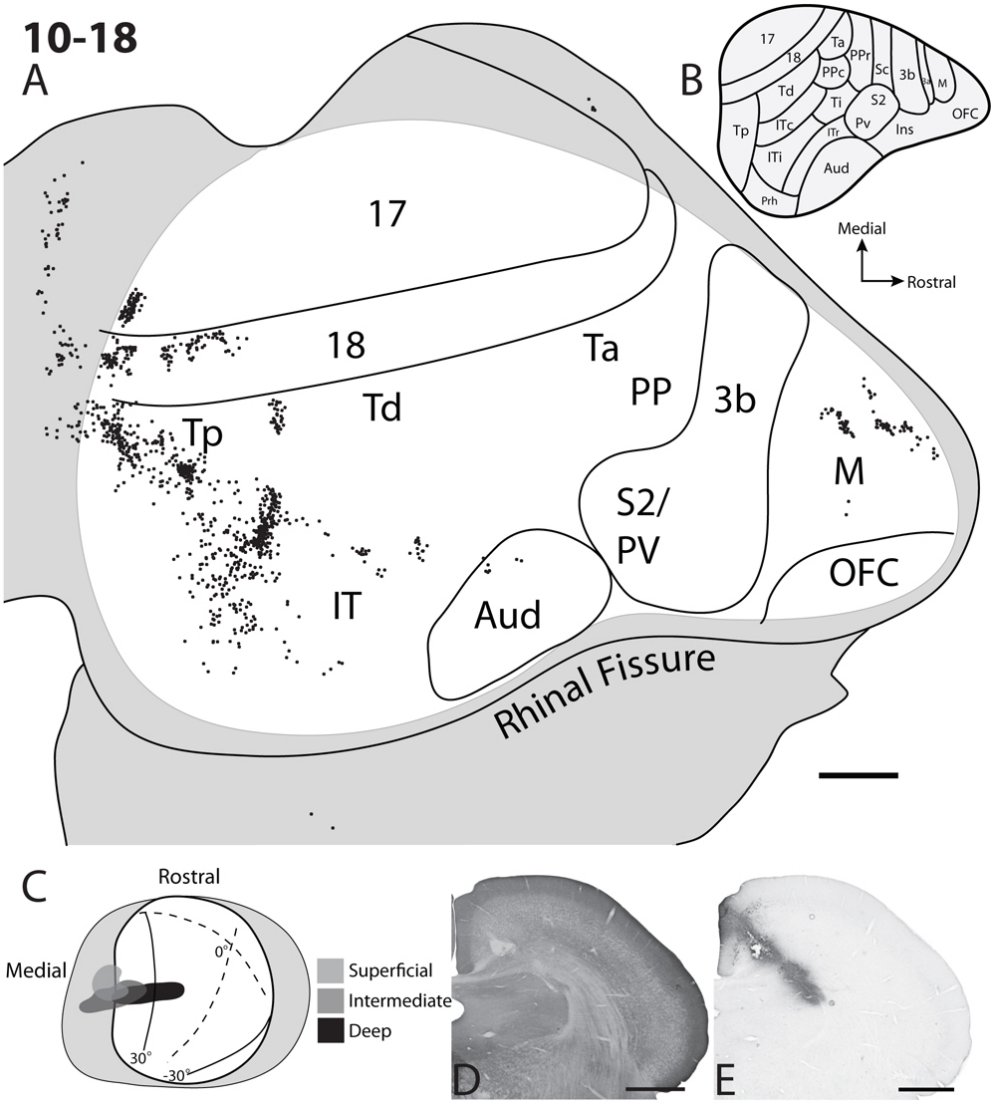
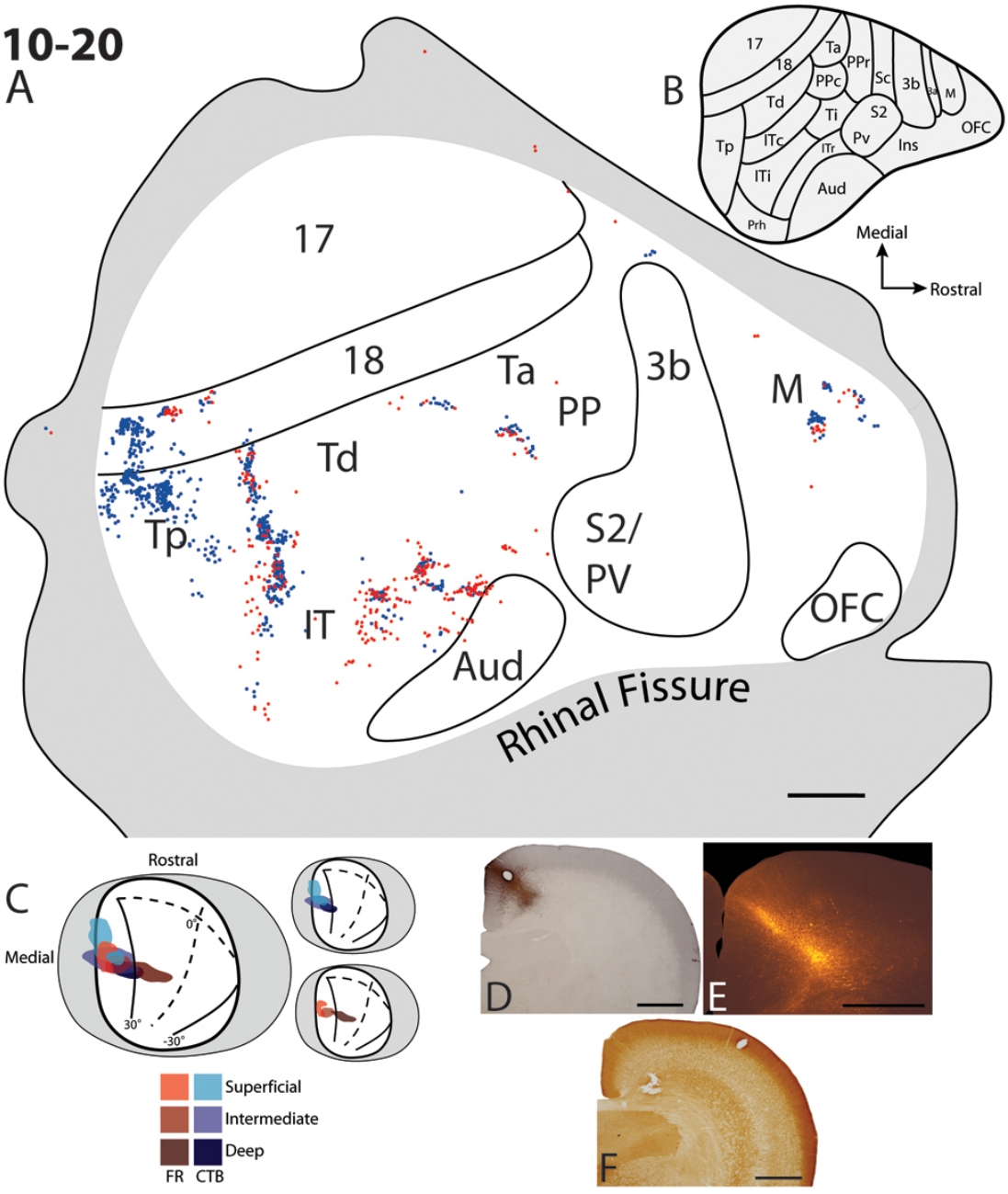


Figure 5.8. *Cortical projections to the superficial, intermediate, and deep layers of the superior colliculus in case 10-18.* ***A****. The distribution of retrogradely labeled cells within the flattened cortex after a cholera toxin subunit B (CTB) injection into the superior colliculus. Black dots represent retrogradely labeled cells, solid lines are borders determined based on myelin stained sections and grey shaded areas represent unfolded cortex along the medial wall and ventral surfaces of the brain.* ***B.*** *Cortical organization map adapted from Wong and Kaas, 2009.* ***C****. Dorsal view reconstruction of the superior colliculus superimposed on the visuotopic map by Lane et al., 1971. Grey area represents the medial and lateral aspects of the superior colliculus unfolded. Darker shades of grey within the superior colliculus represent intermediate and deep layers of the superior colliculus included in the CTB injection site.* ***D****. Photomicrograph of a coronal section stained for CO that is adjacent to* ***E*** *a CTB stained section through the injection site within the superior colliculus. Scale bar for A is 2mm, and D and E is 1mm.*

wall, thus the injection included the medial superficial layers and progressed more laterally into the deeper layers of the superior colliculus. The FR injection site did not include much of the SGS. The lack of CTB cells within striate cortex was surprising given that one of the foci of the injection was within the lower SGS (Fig. 5.9D). Possibly the relevant part of area 17 representing the paracentral upper visual field was lost during flattening and processing. Unlike the previous case (10-18, Fig. 5.8), there does not appear to be a gap in the spread of the CTB injection site across the layers of the superior colliculus, which could be why there were labeled cells within the PPc and Ta region for this case (Fig. 5.9) and not for case 10-18 (Fig. 5.8).

Other, focused patches of labeled cells, representing the parafoveal upper visual field, were present within areas 18, TP, Td; regions within medial IT and within the Ta/PPc region; while a few labeled cells were scattered within the lateral IT region. The more focused patches of label suggest that 18, Tp, Td, and PPc have topographic representations, while the scattering of labeled cells suggests that lateral IT does not contain a topographic representation. As in the cases 09-62 (Fig. 5.4), and 10-18 (Fig. 5.8), multiple patches of label were observed along the length of area 18, suggesting a possible modular characteristic of this area in trees shrews (Sesma et al., 1984). Within the Tp region, multiple clusters of cells were apparent with one dense patch located rostromedially, and a second more diffuse patch located caudolaterally, similar to cases 11-35R (Fig. 5.6) and 10-18 (Fig. 5.8). The two closely spaced patches of labeled cells in Tp were relatively far away from the line of cells observed in Td. The line of labeled cells within Td extended ventrally into IT, with a slight gap between cells in Td and those within the IT region. The more ventral patch of cells could be all within ITc as described

Figure 5.9. Cortical projections to the superficial, intermediate, and deep layers of the superior colliculus in case 10-20. **A**. The distribution of retrogradely labeled cells within the flattened cortex after fluoro-ruby (FR) and cholera toxin subunit B (CTB) injections into the superior colliculus. Solid lines represent borders determined using myelin stained sections. Red dots represent the locations of retrogradely labeled FR cells. Blue dots represent the locations of retrogradely labeled CTB cells. The grey shaded area represents cortex that was either along the medial or ventral surfaces of the brain. **B.** Cortical organization map adapted from Wong and Kaas (2009). **C**. Dorsal view reconstruction of the superior colliculus superimposed with the visuotopic map by Lane et al. (1971) indicating the location of the injection sites. Grey area represents the medial and lateral aspects of the superior colliculus folded out. The red hue areas represent the location of the FR injection site while the blue hue areas represent the location of the CTB injection site. Darker hues indicate the location of the injection sites at deeper levels within the SC. **D.** Photomicrograph of the CTB injection site in a coronal section through the superior colliculus. **E.** Photomicrograph of the FR injection site in a coronal section through the superior colliculus. **F.** An adjacent coronal section to E stained for cytochrome oxidase. The hole in the tissue shows the second CTB injection focus within the SGI of the superior colliculus. Scale bar for A is 2mm, D, E, and F is 1mm.



by Wong and Kaas (2009), within ITi, or another area within IT. Because of the close clustering of CTB labeled cells with FR labeled cells relative to the close positions of the injection sites, it is likely that this IT region has at least a crude topographic organization pattern. Two patches of labeled cells were also located more rostrally within the IT/Ti region, with an additional more rostral patch of labeled cells located close to the border of

auditory cortex. These patches could represent cells within areas Ti, ITi, and ITr (Compare Fig. 5.9A with Fig. 5.9B).

Two patches of label were present within the frontal cortex (Fig. 5.9), similar to case 10-18 (Fig. 5.8). A dense patch of labeled cells was located rostrolaterally, and a more diffuse patch of labeled cells was located more medially. These patches were likely within premotor or prefrontal cortical areas and not within primary motor cortex (Remple et al., 2006, 2007) because of their distance of over 2mm rostral to the area 3b border. No labeled cells were present in orbital frontal cortex, somatosensory, primary motor, or auditory cortex similar to all other cases. Overall, this case provides information on projection patterns of labeled cells to all layers of the superior colliculus. Major differences between this case and the first case (09-62), with similarly placed injection sites, are that labeled cells were observed within frontal cortex, and multiple patches of labeled cells were within area TP. Additionally, the distribution of labeled cells within temporal and parietal areas was denser than that observed for 09-62.

In summary, injections into the superior colliculus revealed corticotectal projecting cells within occipital and temporal visual areas, with multiple focused patches of cells suggesting retinotopic organization patterns within them. However, the consistent pattern of scattered cells throughout much of ventral IT suggests that this region is not retinotopicaly organized. Additionally, injection sites that involved deeper layers of the superior colliculus resulted in labeled cells within frontal cortex, but these labeled cells were likely outside of the primary motor area (Remple et al., 2006; 2007). Labeled cells were only occasionally observed within auditory cortex and the parietal region of cortex including posterior parietal cortex.

5.5. DISCUSSION

In the present study, we examined the areal distribution of cortical areas projecting to the superior colliculus in tree shrews and compared the location of labeled tectal projecting cells with our current understanding of the organization of cortical areas in tree shrew neocortex (Wong and Kaas, 2009). Our injections were placed at different depths of the superior colliculus to reveal differences between projection patterns to the superficial layers of the superior colliculus and those to deeper layers. Additionally, we placed injection sites at various retinotopic locations within the superior colliculus, so that patterns of labeled cells could suggest topographic subdivisions of temporal visual cortex as well as to test if there are differences in cortical projections to different quadrants of the superior colliculus. The results indicate that the majority of cortical cells projecting to the superior colliculus in tree shrews arise from visual or visuomotor cortex. After injections into the superficial layers of the superior colliculus, labeled cells were found in occipital and temporal visual areas, while deeper injections labeled neurons in frontal cortex, likely in prefrontal motor cortex. Our results also provide some insight into the topographic layout of occipital and temporal cortical areas that seems to correlate well with the architectonic subdivisions of cortex described by Wong and Kaas (2009). Finally, few if any, neurons project to the superior colliculus from somatosensory, orbital frontal, primary motor, or auditory cortex.

Some of our results are similar to those reported in a previous study of cortical projections to the superior colliculus (Casseday et al., 1979). Yet, several differences should be noted. For instance, Casseday et al., (1979) reported differences in extrastriate cortical inputs to rostral and caudal aspects of the intermediate layers of the superior colliculus. Their report suggested that labeled cells within extrastriate visual cortex were

found only after injections were placed within the rostral and not caudal portion of the superior colliculus. However, most of our injections were within the caudal superior colliculus, and neurons in extrastriate visual areas were consistently labeled (Figs. 5.4-9). Additionally, Casseday et al. (1979) reported projections from a single area, area 19, which runs along the rostral border of V2 (Figs. 5.1A, 5.10A). However, the multiple patches of labeled neurons along the outer border of V2 for single injections in the present cases is more consistent with the presence of multiple visual areas along the rostral border of area 18, similar to those of previous reports (Sesma et al., 1984; Lyon et al., 1998, Wong and Kaas, 2009). Finally, unlike Casseday et al (1979), we found few, if any, cells projecting to the superior colliculus from somatosensory cortex or the primary motor cortex. These differences in conclusions do not seem to reflect major differences in illustrated results, but rather our current interpretation of the location and organization of somatosensory and motor cortical areas. Thus, a better understanding of the functional organizations of neocortex in tree shrews has allowed us to provide a more accurate view of the projections patterns to the superior colliculus. This information is useful in that it can provide insights on the possible functional characteristics of the superior colliculus in tree shrews, and indicate differences in collicular organization among members of the Euarchontoglires clade. Additionally, our present results help further define the organization of cortical areas in tree shrews by providing support for previously proposed areas and suggesting additional divisions.

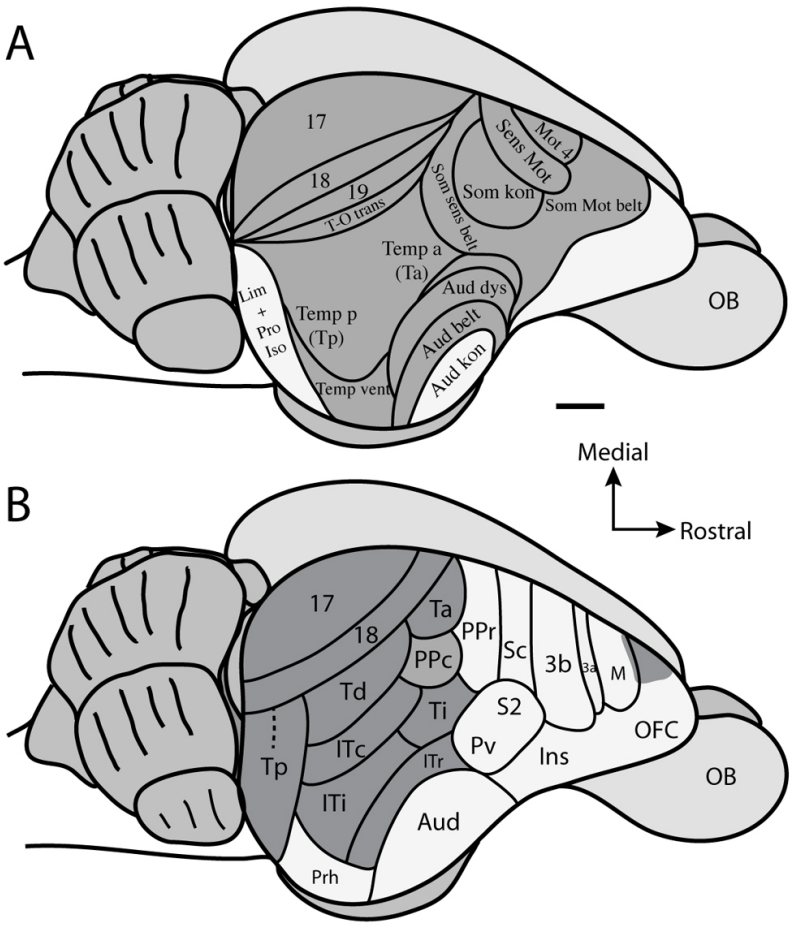


Figure 5.10. *Summary of the locations of cortical areas projecting to the superior colliculus based on the study by Casseday et al., 1979* ***(A)****, and the summary of results from the current study (****B****). See Figure 1 for abbreviations. Scale bar is 2mm.*

5.5.1. Occipital cortical areas

Injections of superficial and intermediate layers of the superior colliculus mainly produced retrogradely labeled cells in visual areas within the occipital and temporal cortex. Neurons in area 17 were only labeled when the SGS was included within the injection site (Figs. 5.4, 5.5, 5.7, and 5.8) and no labeling was observed in area 17 when it was not included (Figs. 5.6, and 5.9). This is consistent with previous experiments indicating that area 17 projects to the SGS as well as the dorsal-most aspect of the SO in tree shrews (Harting and Noback, 1971; Casseday et al., 1979; Huerta et al., 1985). When

area 17 was included in the corticotectal projections, the locations of labeled cells were in close visuotopic register (see Kaas et al., 1972) with the retinotopy of the superior colliculus (Lane et al., 1971).

Area 18 is visuotopically organized in a mirror reversal to that of 17, with the border between 17 and 18 representing the vertical meridian, and the most caudoventral aspect of area 17 and most caudal and rostral aspects of area 18 representing the far periphery (Kaas et al., 1972; Sesma et al., 1984; Lyon et al., 1998). This organization was apparent in the present study, as superior colliculus injections close to the vertical meridian within the upper visual field resulted in retrogradely labeled cells located close to the 17/18 border (Figs. 5.4 and 5.8). Superior colliculus injections located in the peripheral visual field representation resulted in labeled cells located in the most caudal aspect of area 17, as well as along the most rostral border of 18 (Figs. 5.5 and 5.7), locations displaced from the 17/18 border. Also, injections within the upper visual field representations of the superior colliculus resulted in labeled cells within the caudolateral half of area 18, while injections within the lower visual field representation resulted in labeled cells within the most rostromedial half of area 18, consistent with the retinotopic organization of area 18.

Areas 17 (V1) and 18 (V2) are cortical areas that are homologous to areas found in most other mammals (Kaas, 2002). As in tree shrews, projections from occipital areas 17 and 18 to the superior colliculus are present in most other mammals including rodents (Sefton et al., 1981; Olavarria and Van Sluyters, 1982; Harvey and Worthington, 1990; Rhodes et al., 1991), cats (Updyke, 1977), and primates (Fries, 1984; Lock et al., 2003; Collins et al., 2005; Baldwin and Kaas, 2012).

Our results did not provide evidence for an area 19 along the rostral border of area 18. Such an area was described by Casseday et al. (1979), but has not been included in more recent reports on tree shrew cortical organization (Sesma et al., 1984; Lyon et al., 1998, Wong and Kaas, 2009). Architectonic evidence for an area 19 implies the existence of a single, third visual area, V3, along the outer border of area 18. Compelling evidence for such an area V3 exists for primates (See Lyon and Kaas, 2002 for review) and cats (Hubel and Wiesel, 1965; Donaldson and Whitteridge, 1977). However, in tree shrews, several areas appear to be located along the outer border of area 18 (Sesma et al., 1984; Lyon et al., 1998, Wong and Kaas, 2009). Therefore, it is likely that area V3 evolved independently in cats and primates.

5.5.2. Temporal cortical areas

Along the rostral border of area 18, dorsal temporal cortex has been divided into the temporal posterior (Tp), temporal dorsal (Td), and temporal anterior (Ta) areas (Sesma et al., 1984; Lyon et al., 1998; Wong and Kaas, 2009) (Fig. 5.1B). All injections into the superior colliculus in the present study labeled cells within Tp and area Td, with the exception of case 09-62 (Fig. 5.4). Labeled cells within Ta after superior colliculus injections were more variable, though labeled cells were observed after injections involving intermediate layers of the SC (Figs. 5.5, 5.6, 5.7, 5.9), except for case 10-18 (Fig. 5.8) which may have had a gap of tracer spread within the lower SGS and SAI. Previously, Casseday et al. (1979) concluded that projections to the superior colliculus from extrastriate visual cortex terminate in intermediate and deep layers below the SGS, and that such projections terminated mainly within the rostral aspect of the superior colliculus. In the present study, we too found that projections to dorsal temporal cortex

likely terminate within the intermediate layers of the superior colliculus below the SGS. However, our results differ from those of Casseday et al. (1979) in that we found that projections from such areas were present even after our most caudally placed injections into the superior colliculus, suggesting that temporal visual areas project to both rostral and caudal locations within the superior colliculus.

There was extensive overlap between the distribution of cells labeled in the temporal cortex following the injection of retrograde tracers in the superior colliculus, with regions of temporal cortex that are reciprocally connected to the tectorecipient zones of the pulvinar nucleus (Luppino et al., 1988; Lyon et al., 2003; Chomsung et al., 2008; Chomsung et al., 2010). This provides further evidence that large regions of the temporal cortex in tree shrews are devoted to vision. As described for galagos (Glendenning et al., 1975), projections from the tree shrew pulvinar nucleus form two patches of terminals within temporal cortex, potentially defining two relatively large cortical subdivisions. However, the current results suggest that the tectorecipient regions of the pulvinar may in in fact project to multiple subdivisions within temporal cortex. Though it was difficult to determine the exact borders between Tp, Td, and Ta in the present study, the locations of labeled cells were within the general regions of such areas as described in previous studies (Sesma et al., 1984; Lyon et al., 1998; Wong and Kaas, 2009). Therefore, we describe the pattern of labeled cells for each area further below.

5.5.2.1. Ta

Area Ta has weak connections with area 17, but stronger connections with area 18 (Sesma et al., 1984; Lyon et al., 1998), as well as connections with forelimb regions of primary motor cortex (Remple et al., 2007). Thus, Ta has been considered a visuomotor

area and could be part of the posterior parietal motor areas described in primates (Stepniewska et al., 2005; Gharbawie et al., 2011). In the present study we found that Ta had few projections to the superior colliculus relative to the projections from Tp and Td, and that these projections likely terminate within the intermediate layers of the superior colliculus. Because of the weak projections to the superior colliculus, Ta likely only has weak influences on the superior colliculus functions relative to areas Tp and Td.

5.5.2.2. Td

We found projections from Td to the superior colliculus in all cases except case 09-62 (Fig. 5.4). The location of labeled neurons in Td after variably located injections in the superior colliculus provide evidence that Td is retinotopically organized because labeled cells wee focused in patches; and when separate injections into the superior colliculus were made in non-overlapping locations, separate, non-overlapping patches of cells were located within Td (Fig. 5.5). However, because it is difficult to align results across cases, we are not completely confident in the organization of the retinotopic pattern. Previous reports suggest that the upper visual field is represented caudolaterally and the lower visual field is represented rostromedially (Sesma et al., 1984; Chomsung et al., 2010). Previous reports have also suggested that the visuotopic organization of Td relative to Tp is in a serial repeating pattern, meaning that there is no reversal in the visuotopy across the Tp/Td border. Our present results are consistent with this hypothesis, as the distance between the most mediodorsal patch of cells within Tp was consistently displaced by 2mm from the patch of labeled cells within Td (Figs. 5.5-9).

Td is located along the border of the middle aspect of area 18, has strong connections with area 17, and stains moderately for myelin. Because of these

characteristics, Td has been suggested to be similar to the middle temporal area in primates (Sesma et al., 1984; Lyon et al., 1998). However, MT in primates is displaced rostrally from the area 18 border and Td is not. These and other differences do not support the conclusion that Td is homologous to MT in primates (Kaas and Preuss, 1993).

Our Td connections with the superior colliculus do not provide evidence for or against Td being a homologue of MT because connections between MT and the superior colliculus in primates is variable across primate species, with strong connections observed in New and Old World monkeys (Graham et al., 1979; Fries, 1984; Lock et al., 2003; Collins et al., 2005), but weak or no connections between MT and the superior colliculus observed in galagos (Baldwin and Kaas, 2012).

5.5.2.3. Tp

Tp is located along the most caudolateral aspect of the rostral border of area 18. Multiple patches of label were observed within the region of Tp after single tracer injections into the superior colliculus suggesting that Tp is comprised of more than one retinotopically organized area or is modularly organized. Previous reports show that Tp shares connections with area 17, and 18, with connections between area 18 and Tp being denser (Sesma et al. 1984; Lyon et al., 1998). Sesma et al. (1984) also suggested that Tp may be composed of two visual areas, but architectonic characteristics of two separate areas have not been identified (Sesma et al., 1984; Lyon et al., 1998; Wong and Kaas, 2009).

In case 03-34 (Fig. 5.5), only single patches of labeled cells for single injections sites were present within the region of Tp, and for case 11-35 where two patches were present, the orientation of those patches was different from that observed in other cases.

Thus, the second patch observed for this case may be a result of area 17 contamination. However, in cases 11-35R (Fig. 5.6) and 10-18 (Fig. 5.8), two clearly distinct patches of labeled cells were present within the Tp region after single CTB injections; while the less distinct pattern of labeled cells for case 10-20 (Fig. 5.9) also suggests multiple patches with Tp. Therefore, it is likely that Tp does consist of two topographically organized areas. However, we are not certain of the topographic organization of these areas. For cases 03-34 (Fig. 5.5) and 11-35L (Fig. 5.7) where single patches were present along an axis parallel to the V2 border, the injection sites were very caudal within the superior colliculus. In contrast, cases with more rostral superior colliculus injections resulted in patches of labeled cells that were more spread apart within Tp (Figs. 5.6, 5.8, and 5.9) suggesting that there could be a reversal between the two domains within Tp at the representation of peripheral vision.

In cases 11-35L (Fig. 5.7) and 11-35R (Fig. 5.6), where injections were placed iontophoretically, labeled cells were present ventral to the patches of labeled cells described in dorsal Tp close to the rostral border of area 18. Label in this location was only present in these two cases, and may be a result of cortical contamination of area 17 during tracer placement into the superior colliculus, or could be a result of injections into more lateral aspects of the superior colliculus. But this was not apparent in case 03-34 (Fig. 5.5) with a lateral peripheral injection.

There does not appear to be a reversal in topography between the Tp/Td border, as patches of label were consistently separated from one another by 2 to 3mm regardless of the injection site location within the superior colliculus. A lack of a reversal between Td and Ta also seems likely. Thus, these consistent separations between patches within

Tp, Td, and Ta regardless of injection placement may suggest that these three areas share a serial topography similar to descriptions by Chomsung et al. (2010).

5.5.3. Inferior temporal cortical areas

In early architectonic studies, the inferior temporal cortex was not subdivided (Zilles et al., 1978). More recently, IT cortex has been subdivided into three areas by Remple et al. (2007) based on differences in connections with cortical motor areas and by using architectonic analysis. Wong and Kaas (2009) defined four subdivisions, which are the inferior temporal cortex (IT), caudal IT (ITc), inferior IT (ITi), and rostral IT (ITr), along with another cortical area, the temporal inferior area (Ti). We found labeled cells within all of these areas after injections that involved intermediate layers of the superior colliculus, similar to results of Casseday et al (1979). Our current results suggest that at least four divisions exist in IT cortex. Multiple isolated patches of labeled cells were present within different locations after single injections in the superior colliculus (Figs. 5.5, 5.6, 5.7, and 5.9). Injections in more peripheral locations produced label that was spatially close together within the center of IT (Fig. 5.5), while more rostral injections produced label at more distantly displaced locations (Figs. 5.6, 5.8, and 5.9).

ITc and ITi are thought to be more visual in function (Wong and Kaas 2009), while ITr may be more associated with auditory processing (Oliver and Hall, 1978; Wong and Kaas, 2009); but ITi also shares connections with motor cortex (Remple et al., 2007).

Our present results combined with results from Wong and Kaas (2009) suggest that there likely is a division running perpendicular to the rostral border of our architectonically defined auditory region that separates areas ITi from Ti. Along the line

of decussation it appears that more peripheral vision is represented with central vision moving outward (See Fig. 5.5 and 5.9).

In the present study, we consistently observed a strong band of labeled cells just ventral to cells within Td, which was likely within ITc as described by Wong and Kaas (2009). In most cases, this patch of label was separated from cells within Td (Figs. 5.6, 5.7, 5.8 and 5.9), but in other cases these patches seemed to run closely to one another (Fig. 5.5). Finally, labeled cells within ventral IT were diffusely scattered for all cases, suggesting that this area does not have a visuotopic organization pattern.

5.5.4. Posterior parietal cortex

In the present report, it was difficult to define the exact border of the parietal regions because of the difficulty to differentiate between PPc and Ti using CO and myeloarchitecture. Projections from the parietal cortical region such as PPc were present in three cases (Figs. 5.5, 5.6, and 5.7), yet the label was very weak relative to temporal and even frontal cortical areas. Similar findings are also observed in Casseday et al. (1979). In primates, strong projections from the posterior parietal area are observed in galagos (Baldwin and Kaas, 2012), New World (Collins et al., 2005), and Old World monkeys (Fries, 1984, Lock et al., 2003). The difference in projections from posterior parietal areas in primates relative to the closely related tree shrew may reflect specializations within the motor networks of primates, who have a much more expanded parietal cortex relative to tree shrews. Not much is currently known about the projection pattern of posterior parietal cortex in rodents.

5.5.5. Somatosensory cortex

We found few, if any, cells within somatosensory areas for any of our cases, which is in contrast to Casseday et al. (1979). It is important to note that Casseday and colleagues defined cortical areas based on Nissl staining, as well as descriptions of cortical areas prior to 1979 including those based on anatomical connection studies of the LGN, pulvinar, and MGN (Diamond et al., 1970; Harting et al., 1973; Casseday et al., 1976; Oliver and Hall, 1978), and on other cortical organization experiments within tree shrews (Snyder and Diamond, 1968). However, many interpretations of the organization of cortex in tree shrews described in Casseday et al. (1979) have since changed (compare Fig. 5.1A to 5.1B). If one were to superimpose the map of Wong and Kaas (2009) onto the summary diagrams of label for cases with intermediate and deep injections in Casseday et al. (1979), some of the labeled cells would be within primary somatosensory, primary motor cortex, and within the S2/PV areas (See Figs. 10 and 11 of Casseday et al., 1979). In the Casseday et al. (1979) study, labeled cells were observed within the S2/PV region of today's schemes of cortical areas in tree shrews (Wong and Kaas, 2009) after injections within intermediate layers of the superior colliculus; and labeled cells were found within dorsal somatosensory and motor areas after deeper injections within the superior colliculus. Also, the injections into the superior colliculus that resulted in the most labeled cells within somatosensory and motor cortex of Casseday et al. (1979) were made into a more lateral location within the superior colliculus than any of our own cases (Fig. 5.10), at least for our injections that involved all layers of the superior colliculus. However labeled cells within somatosensory and motor cortex were also observed with

more medial injections (Tupaia 0-197 Fig. 10 of Casseday et al., 1979), and that injection site location does match sites within our own cases.

One possible reason for these differences could be in the way injections were made, with Casseday et al. (1979) placing tracers through the cerebellum and brainstem through the inferior colliculus and into the superior colliculus horizontally, while our tracers were placed vertically into the superior colliculus after cortical aspiration, retraction, or through other visual cortical areas. It could be that the label observed in Casseday et al. (1979) is a result of contamination of other brainstem structures with connections to somatosensory areas.

Our results also differ from reports in rodents where projections from whisker fields in primary and secondary somatosensory are prominent (Wise and Jones, 1977; Rhoades et al., 1981). The present results in tree shrews are similar to results from primates (Fries, 1975; Collins et al., 2005; Baldwin et al., 2012), where few cortical inputs originate from primary somatosensory areas. However, in primates, S2/Pv has been reported to project to the deep layers within the superior colliculus (Collins et al., 2005; Wu et al., 2005; Baldwin et al., 2012).

The lack of evidence for connections between somatosensory cortical areas and the superior colliculus in the present study may reflect technical influences noted above.

5.5.6. Auditory cortex

Auditory cortex of tree shrews contains at least core and belt regions, both staining heavily for myelin (Oliver and Hall, 1975; Casseday et al, 1976; Oliver and Hall, 1978; Wong and Kaas, 2009). In the present report we combined both belt and core areas into a single auditory region (Aud). Few cells were found in auditory cortex after either

superficial or deep injections. Casseday et al. (1976, 1979) reported projections from the auditory core to the inferior colliculus, but no projections to the superior colliculus. However, auditory belt and auditory dysgranular regions did project strongly to intermediate and deep layers of the superior colliculus. We believe that the projections from Casseday et al. (1979) are likely to be from ITr and thus are consistent with our present results.

In primates, few projections arise from the auditory core, belt, or parabelt regions (Fries, 1984; Lock et al., 2003; Collins et al., 2005; Baldwin et al., 2012). Yet there are projections to the superior colliculus from a region between MST and auditory cortex, known as the temporal parietal area (Tpt), which is thought to be involved in auditory processing as well as have connections between posterior parietal and frontal areas (Preuss and Goldman-Rakic, 1991; Stepniewska et al., 2009).

5.5.7. Motor and Frontal cortex

We found labeled cells within frontal cortex after injections that involved deep layers of the superior colliculus. In previous reports, primary motor cortex has been divided into M1 and M2 regions (Remple et al., 2006, 2007; Wong and Kaas 2009) based on differences in connections, stimulation thresholds, and architecture. In the present report we combined M1 and M2 into a single motor area, M. Two patches were consistently observed with one patch located more rostromedial than the other (Figs. 5.5-7). Though the most caudal patch of cells in frontal cortex for case 10-18 (Fig. 5.8) and 10-20 (Fig. 5.9) could be within the motor cortex, it is likely the labeled cells lie within premotor and prefrontal cortex. Electrophysiological and architectonic analysis of motor cortex by Remple et al. (2006, 2007) indicates that the most rostral border of M2 is 2mm

away from the rostral border of area 3b. Labeled cells in this study were consistently located more than 1.5mm away from the rostral 3b border. Our results are similar to those of Casseday et al. (1979), when considering the location of labeled cells in that study with our current understanding of cortical organization. Also, few connections were observed in cortex rostral to motor cortex (Remple et al., 2007). It could be that one patch of labeled cells is within an eye or gaze movement region, as such gaze fields are present within this region of rodents (Hall and Lindholm, 1974; Donahue and Wise, 1982; Neafsey et al., 1986; Rapisarda, 1990; Stuesse and Newman, 1990; Tsumori, 2001). However, such a field was not observed in the microstimulation experiments of Remple et al. (2006), and it is unlikely that this field would be similar to FEF where saccadic movements are elicited as tree shrews are not expected to foveate given their less specialized retina (Samorajski et al., 1966).

In rodents, direct projections from motor areas such as the barrel motor cortex to the superior colliculus are present (Miyashita et al., 1994). However, in primates few if any corticotectal projections arise from primary motor areas (Collins et al., 2005; Fries et al., 1984; Baldwin et al., 2012). While there are strong tectal projections from the frontal eye fields in New and Old World monkeys (Collins et al., 2005; Fries, 1984), prosimian primates have weaker frontal eye field projections with stronger projections arising from other prefrontal areas (Baldwin and Kaas, 2012).

5.5.8. Conclusions

The superior colliculus (tectum) is found in all vertebrates; however, the specific function of this structure may vary depending on differences in afferent and efferent connections. The neocortex can influence the functional properties of the superior

colliculus through projections to the superior colliculus, which then in turn projects to motor centers and the visual thalamus.

Because of their phylogenetic position, tree shrews provide important information for comparing brain organization patterns in primates and rodents. Our present results provide evidence that cortical visual and visuomotor inputs influence the superior colliculus in tree shrews more so than cortical inputs from the somatosensory, auditory, or motor cortices. These findings are more congruent with observations in primates than rodents. In primates, the inputs to the superior colliculus are more visual in nature and do not contain many inputs from somatosensory, auditory, or motor areas (Fries, 1984; Lock et al., 2003; Collins et al., 2005; Baldwin and Kaas, 2012). In contrast, projections to the superior colliculus from somatosensory cortex, primarily from the barrel fields, are observed in rodents (Wise and Jones, 1977; Rhoades, 1981; Olavarria and Van Sluyters, 1982; Cadusseau and Roger, 1985; Welker et al., 1988; Harvey and Worthington, 1990; Hoffer et al., 2005; Aronoff et al., 2010; etc.).

17	Area 17
18	Area 18
19	Area 19
3b	Primary somatosensory area
AChE	Acetylcolinesterase
Aud	Auditory cortex
Aud Kon	Auditory konio cortex
Aud Belt	Auditory belt
Aud Dys	Auditory dysgranular cortex
CO	Cytochrome oxidase
IT	Inferior temporal cortex
Lim	Limbic cortex
Mot 4	area 4 of motor cortex
M	motor cortex
OFC	orbital frontal cortex
Pro Iso	proisocortex
PV	parietal ventral area
S2	secondary somatosensory area
SC	superior colliculus
Sens mot	sensory-motor cortex
Sens kon	somatic koniocortex
Som sens belt	somatic sensory belt

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CHAPTER 6

CORTICAL PROJECTIONS TO THE SUPERIOR COLLICULUS IN GRAY SQUIRRELS (*SCIURUS CAROLINENSIS*)

6.1. ABSTRACT

The superior colliculus (also called the tectum) is an important midbrain structure involved with integrating information from varying sensory modalities and sending motor signals to produce orienting movements toward environmental stimuli. Because of this role, the superior colliculus receives a multitude of sensory inputs from a wide variety of subcortical and cortical structures. Proportionately speaking, the superior colliculus of gray squirrels is among the largest of all studied mammals, suggesting the importance of this structure in the behavioral characteristics of gray squirrels. Yet, our understanding of the connections of the superior colliculus in gray squirrels is lacking, especially with respect to the possible cortical influences. In the present study, we placed anatomical tracer injections within the medial wall of the superior colliculus of three gray squirrels (*Sciurus carolinensis*) and analyzed the areal distribution of corticotectal projecting cells in flattened cortex. Our results indicate that the superior colliculus receives cortical projections from a number of visual, higher order somatosensory, and higher order auditory regions, as well as limbic, retrosplenial, and anterior cingulate cortex. Few, if any, corticotectal projections originate from primary motor, primary somatosensory, primary auditory cortex, or parietal cortical regions. This distribution of inputs is similar to the distribution of inputs described in other rodents such as rats and mice, yet the lack

of inputs from primary somatosensory and motor cortex are features of corticotectal inputs more similar to those observed in tree shrews and primates and could reflect a behavioral shift from somatosensory (vibrissae) to visual environmental navigation.

6.2. INTRODUCTION

Of all studied mammals, gray squirrels have one of the largest superior colliculus, with a surface area that can be ten times larger than that of the rat with a similar body size (Le Gros Clarke, 1959; Lane et al., 1971; Kaas and Collins, 2001). Since the superior colliculus is important for integrating sensory stimuli into motor commands, the large superior colliculus of gray squirrels is likely highly influential in this species' perceptual and behavioral characteristics. This integrating process incorporates incoming subcortical sensory inputs from the retina (Cusick and Kaas, 1982); subcortical auditory structures including the external inferior colliculus, the brachium of the inferior colliculus, and the lateral lemniscus (Druga and Syka, 1984; Baldwin et al., 2011); and somatosensory inputs from the brainstem and spinal cord (cat: Edwards et al., 1979; monkey: Wiberg et al., 1987), as well as information from sensory cortex (May, 2006; Gould et al., 1989). However, our understanding of the cortical influences on the superior colliculus in this species is lacking. The architectonic subdivisions of the gray squirrels are distinct and have been well studied (Kaas et al., 1972; Merzenich et al., 1976; Sur et al., 1978; Nelson et al., 1979; Krubitzer et al., 1986; Kaas et al., 1989; Luethke et al., 1988; Wong et al., 2008; Wong and Kaas, 2008; Cooke et al., 2011), providing a basis for examining the cortical influences on the superior colliculus of gray squirrels.

The goals of the current study were to determine the areal distribution of corticotectal projecting cells in gray squirrels by placing anatomical tracer injections into the superior colliculus and to analyze the distribution of tectal projecting cells throughout cortex. In analyzing the inputs to the superior colliculus, we were also interested in how such cortical influences observed in gray squirrels compare with corticotectal inputs

found in other member species of the Euarchontoglires clade, which includes rodents, such as squirrels, rats, and mice, as well as tree shrews, and primates. By comparing our results with those of other species within the Euarchontoglires clade, we can gain important insights on possible evolutionary changes that may have occurred in the function of the superior colliculus between non-primates and primates, as well as assess possible specializations of the superior colliculus function between arboreal diurnal species and ground dwelling nocturnal species.

6.3. MATERIALS AND METHODS

Corticotectal projections were analyzed in three gray squirrels. The methods used for the current study have been described previously (Baldwin et al., 2011). All surgical procedures were conducted under aseptic conditions and were in accordance to an approved protocol under the Vanderbilt University Institutional Animal Care and Use Committee and adhered to NIH guidelines.

6.3.1. Surgical procedures and tracer placement

Animals were initially given an intramuscular injection of a mixture of ketamine (120mg/kg) and xylazine (8 mg/kg). Once anesthetized, their heads were shaved, and their eyes were protected with eye lubrication. A small amount of lidocaine was injected under the midline of the scalp, as well as within the ears. Heads were then secured in a stereotaxic instrument, and animals were maintained under anesthesia using isoflurane gas (0.5-2%) through a facemask. Heart rate, body temperature, as well as oxygen and carbon dioxide levels were monitored throughout the entire surgical procedure. When a steady level of anesthesia was maintained and animals were non-reflexive in response to a gentle pinch, a midline incision of the scalp was made and the skin was retracted to reveal the left caudal half of the skull. A craniotomy was then made, and the dura was reflected to expose the left occipital lobe. Brain tissue overlying the left superior colliculus was removed by suction, and the blood vessels between the left and right hemispheres as well as the medial wall of the right cortical hemisphere were retracted in order to visualize the medial wall of the right superior colliculus (Fig 6.1B). Once exposed, anatomical tracers including 0.4-0.8µl of Fluoro ruby (FR: Molecular Probes Invitrogen, Carlsbad, CA; 10% in distilled water), and 0.4-0.6µl of Cholera toxin subunit

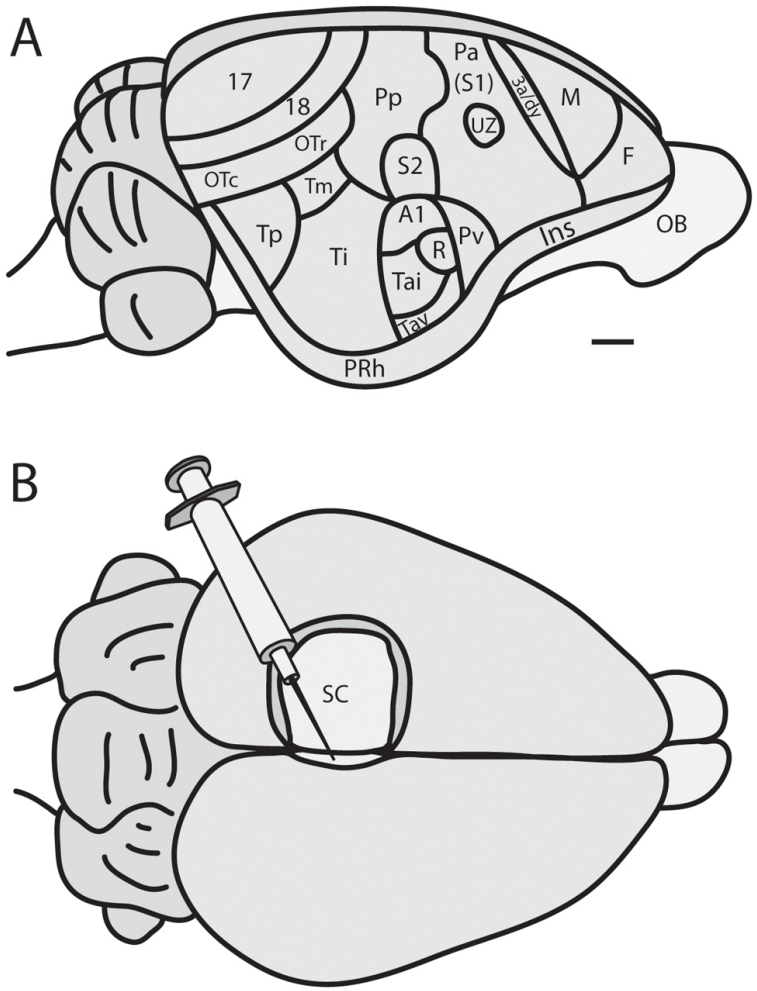


Figure 6.1. Cortical organization of gray squirrels and methodological procedures. ***A****. Depicts the organization of the squirrel temporal cortex. Borders are adapted from previous descriptions of Wong and Kaas (2008).* ***B****. The method used to access the superior colliculus for tracer injections. Portions of the occipital and parietal cortex were removed by aspiration, and the intact hemisphere was slightly retracted in order to reveal the underlying colliculus for tracer placement under visual guidance. Scale bar is 5mm.*

B (CTB: Molecular Probes Invitrogen; 10 % in distilled water) were injected into the right superior colliculus using a Hamilton syringe with an attached, beveled glass tip. Any leakage of tracer outside the target area during placement was removed with saline rinses and cotton swabs. Once tracers were placed, the aspiration lesion from the left cortical hemisphere was filled with gelfoam. The dura was replaced and the opening was

sealed with a dental cement cap. The skin was then sutured and animals were taken off anesthetic gas. When animals became mobile during post surgical recovery, they were given Ketoprofen (2.2 mg/kg, intramuscular) analgesic, placed back in their home cage with food and water, and were continually monitored for three to seven days.

6.3.2. Histological procedures and data analysis

Three to seven days after tracer placement, animals were given an initial intramuscular injection of ketamine/xylazine (Ket: 1120 mg/kg Xyl: 8 mg/kg) followed by a lethal does of sodium pentobarbital (80 mg/kg: intraperitoneal). They were then perfused with phosphate buffered saline (PBS; pH 7.4), followed by 2% paraformaldehyde (para), followed by a mixture of 2% para with 10% sucrose. Brains were then removed. The cortex was separated from the brainstem and thalamus, and artificially flattened and placed in a 30% sucrose solution made in phosphate buffer. The brain stem and thalamus were placed in 4% para for 2 to 6 hours and then placed in 30% sucrose solution made in phosphate buffer. Both cortical and subcortical tissue blocks were then stored in the cold room for twenty four to forty eight hours.

Cortex was cut into a series of three or four in a plane parallel to the pia, while the brainstem and thalamus were cut in the coronal plane in series of four or five. One series of each tissue block was processed for CTB label using the protocol described in Baldwin et al., 2011. A second series was mounted directly onto glass slides for analysis of FR label. The remaining cortical series were processed for myelin (Gallyas, 1979) or cytochrome oxidase (CO: Wong-Riley, 1979). The remaining brainstem and thalamus sections were processed for CO, acetylcholinesterase (AChE: Geneser-Jensen and Blackstand, 1973), or other anatomical markers not described in this report.

Retrogradely labeled cortical cells were plotted using an X-Y plotter (Neurolucida systems MicrobrightField, Williston, VT), and plots of labeled cells were aligned to adjacent sections stained to reveal areal borders within cortex. All sections with retrogradely labeled cells were aligned to each other and architectonic sections using common blood vessels and local features in Adobe Illustrator. Brainstem sections containing the injection sites were aligned to adjacent anatomical sections in order to determine the depth of the injection site within the superior colliculus. Dorsal view reconstructions of the injection sites were created by projecting the location and spread of the injection sites onto a topographic map of the superior colliculus from Lane et al. (1971). Digital images of sections were taken using a DXM1200F digital camera mounted to a Nikon E800S microscope (Nikon, Inc., Melville, NY). Digital images were unaltered except for brightness and contrast adjustments using Adobe Photoshop.

6.3.3. Identifying injection site locations

The superior colliculus of gray squirrels can be divided into seven main layers with some layers having multiple sublayers (see Baldwin et al., 2011) (Fig. 6.2A). The superficial layers consist of the stratum zonale (SZ), the stratum griseum superficiale (SGS), and the stratum opticum (SO). These layers have been associated with visual functions, while the deeper layers are associated with multisensory and motor functions (for review see May, 2006). These deeper layers consist of the stratum griseum intermediate (SGI), which is has three sublayers (SGI_a, SGI_b, and SGI_c); the stratum album intermediate (SAI); the stratum griseum profundum (SGP); and the stratum album

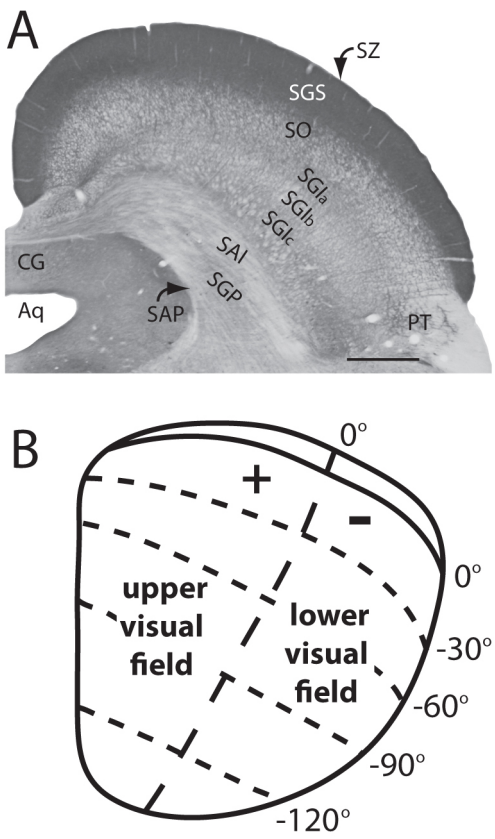


Figure 6.2. The superior colliculus of the gray squirrel. ***A.*** *Coronal view of the gray squirrel superior colliculus stained for cytochrome oxidase. Seven main layers within the superior colliculus can be visualized such as the stratum zonale (SZ), stratum griseum superficial (SGS), stratum opticum (SO), stratum griseum intermediate (SGI), stratum album intermediate (SAI), stratum griseum profundum (SGP), and the stratum album profundum (SAP). The SGI can be further subdivide into three layers (a, b, and c) and the SGS is often subdivided as well.* ***B.*** *Retinotopic organization across the dorsal view of the superior colliculus as determined by Lane et al. (1971). Scale bar for A is 1mm.*

profundum (SAP). All of these layers, and sublayers can be identified using CO, Nissl, AChE, and VGLUT2 staining procedures (Baldwin et al., 2011).

The retinotopy of the superior colliculus was determined in an electrophysiological mapping experiment by. (1971). The upper field is represented medially, and the lower visual field is represented laterally with central vision represented rostrally and peripheral vision represented caudally within the superior

colliculus. In the present study, we inferred the topographic location of the injection sites by superimposing the retinotopic map of Lane et al. (1971) (Fig. 6.2B) on top of our dorsal view reconstructions of the superior colliculus, and therefore, our location sites are general estimates of the actual topographic locations and are not exact. All results in the current report are from injections placed into the upper visual field representation of the superior colliculus.

6.3.4. Identifying the location of labeled cells

The cortical organization of the gray squirrel has been described previously by a number of studies (Hall et al., 1971; Merzenich et al., 1976; Sur et al., 1978; Nelson et al., 1979; Krubitzer et al., 1986; Luethke et al., 1989; Kaas et al., 1989; Wong and Kaas, 2008; Cooke et al., 2011). In the present study, cortical tissue was cut parallel to the pial surface after the brains had been mechanically flattened (Fig. 6.3). In such preparations, primary cortical areas were easily identified because of their dark myelin and CO staining (Wong and Kaas, 2008); however, the borders of other areas were somewhat more difficult to determine.

Areas 17 and 18 are obvious in myelin stained sections in flattened tissue, with area 17 staining darkly for myelin and area 18, along the lateral border of area 17, staining moderately for myelin (Krubitzer et al., 1986; Luethke et al., 1988; Kaas et al., 1989; Wong and Kaas, 2008). Other dark myelin staining areas include area 3b/S1, Tp, Tm, and auditory cortex (Aud). Aud is composed of multiple auditory fields (Merzenich et al., 1976; Luethke et al., 1988), but we do not make such distinctions in the present report. Motor cortex (M) also stains moderately for myelin (Wong and Kaas, 2008; Cooke et al., 2012). Adjacent to Tp, is Tm, which stains less darkly for myelin than Tp,

but more darkly than surrounding cortex (Fig. 6.3). Other cortical areas that were apparent in our architecture sections included the unresponsive zone (UZ). UZ is positioned within area 3b/S1, but stains weakly for myelin. Cortical areas that were less apparent in our architecture sections were estimated based on the locations of such areas relative to known borders as described in previous reports (Wong and Kaas, 2008).

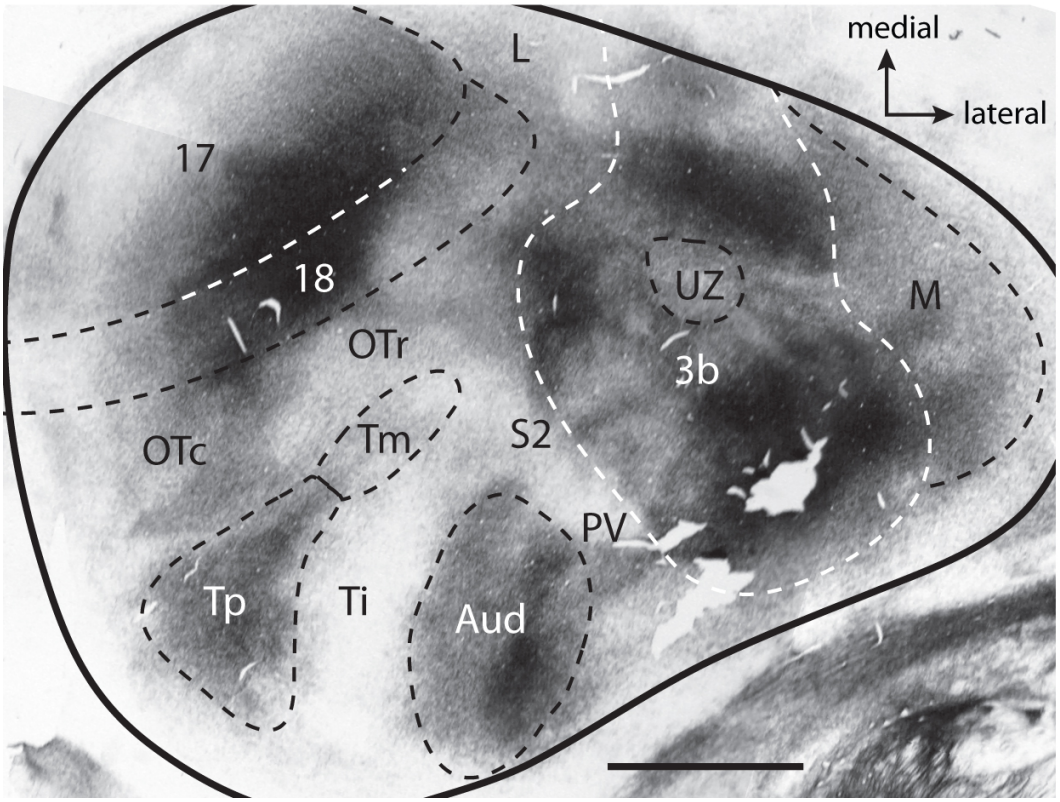


Figure 6.3. Myeloarchitecture of cortical fields of a brain section cut parallel to the pial surface. Areas 17, Tp, Auditory (Aud), and 3b, stain darkly for myelin. Other areas that stain moderately for myelin are areas 18, Tm, and motor cortex (M). The unresponsive zone (UZ) is centered within area 3b and stains lightly for myelin. Though OTc stains slightly darker for myelin than OTr, it is difficult to determine the border between caudal and rostral regions of occipital-temporal cortex and therefore their labels are merely placed in the general regions. The limbic region stains lightly for myelin compared to area 17 and 18. Scale bar is 5mm.

6.4. RESULTS

Corticotectal projections were studied in three gray squirrels using injections of CTB and FR anatomical tracers into the superior colliculus. The locations of retrogradely labeled cells were analyzed from cortical sections cut parallel to the pial surface after the cortex had been flattened. We first present data on the location of the injection sites, and then present data on the pattern of labeled cells within different areas and regions of gray squirrel cortex.

6.4.1. Injection site locations

All cases contain injections that involve the upper visual field representation of the superior colliculus (Figs. 6.4-6). Injection cores for the CTB and FR injections in case 09-50 (Fig. 6.4) involve the lower SGS, all of SO, and upper portions of the SGI layers of the superior colliculus (Fig. 6.4D and F). The FR injection core for case 11-42 (Fig. 6.5) was more superficial than both injections sites for case 09-50 as it involves the SO, as well as both upper and lower portions of the SGS. The CTB injection core is centered within the middle of the SGI, but the core includes the SO, portions of the SGS, and even the upper portion of the SAI. Finally, the injection sites for the third case, 11-37 (Fig. 6.6), are located much deeper within the superior colliculus than the previously mentioned cases. In case 11-37, the FR injection core (Fig. 6.6E) is centered at the lower aspect of the SGI and with the tracer spread including the full width of the SGI and the SO. The CTB injection for case11-37 (Fig. 6.6D) includes all layers of the superior colliculus.

6.4.2. Patterns of labeled cells in cortex

Labeled cells were present throughout a number of cortical visual areas, as well as retrosplenial, limbic, cingulate, and somatosensory cortical areas (Figs 6.4-6). The presence of labeled cells depended on the depth of the injection sites, with more superficial superior colliculus injections resulting in labeled cells in early visual and temporal visual cortical areas; while deeper injections resulted in labeled cells in the retrosplenial, limbic, cingulate, and somatosensory areas including parts of the insula. More specific descriptions of labeled cells within regions and areas follow.

6.4.2.1. Areas 17 and 18

Labeled cells were only present within area 17 when the injection site included upper portions of the SGS, such as for the FR and CTB injections in case 11-42 (Fig. 6.5). These cells spanned the caudal aspect of area 17 with the majority of FR cells located more lateral to the CTB labeled cells, which is consistent with the visuotopy of area 17 in squirrels where the upper visual field is represented caudally and peripheral vision represented medially, away from the area 17/18 border (Hall et al., 1971). The large spread in the FR cells along the caudal aspect of area 17 likely reflects the rostral/caudal spread of the FR tracer within the superior colliculus along the medial wall. Only a few CTB labeled cells are located within area 17 for this case (Fig. 6.5). A few scattered FR labeled cells were present within area 17 of case 09-50 (Fig. 6.4), as were a few CTB labeled cells for case 11-37 (Fig. 6.6). The retinotopic location of the few labeled cells in case 11-37 (Fig. 6.6) did correspond to the location of the injection site in the superior colliculus.

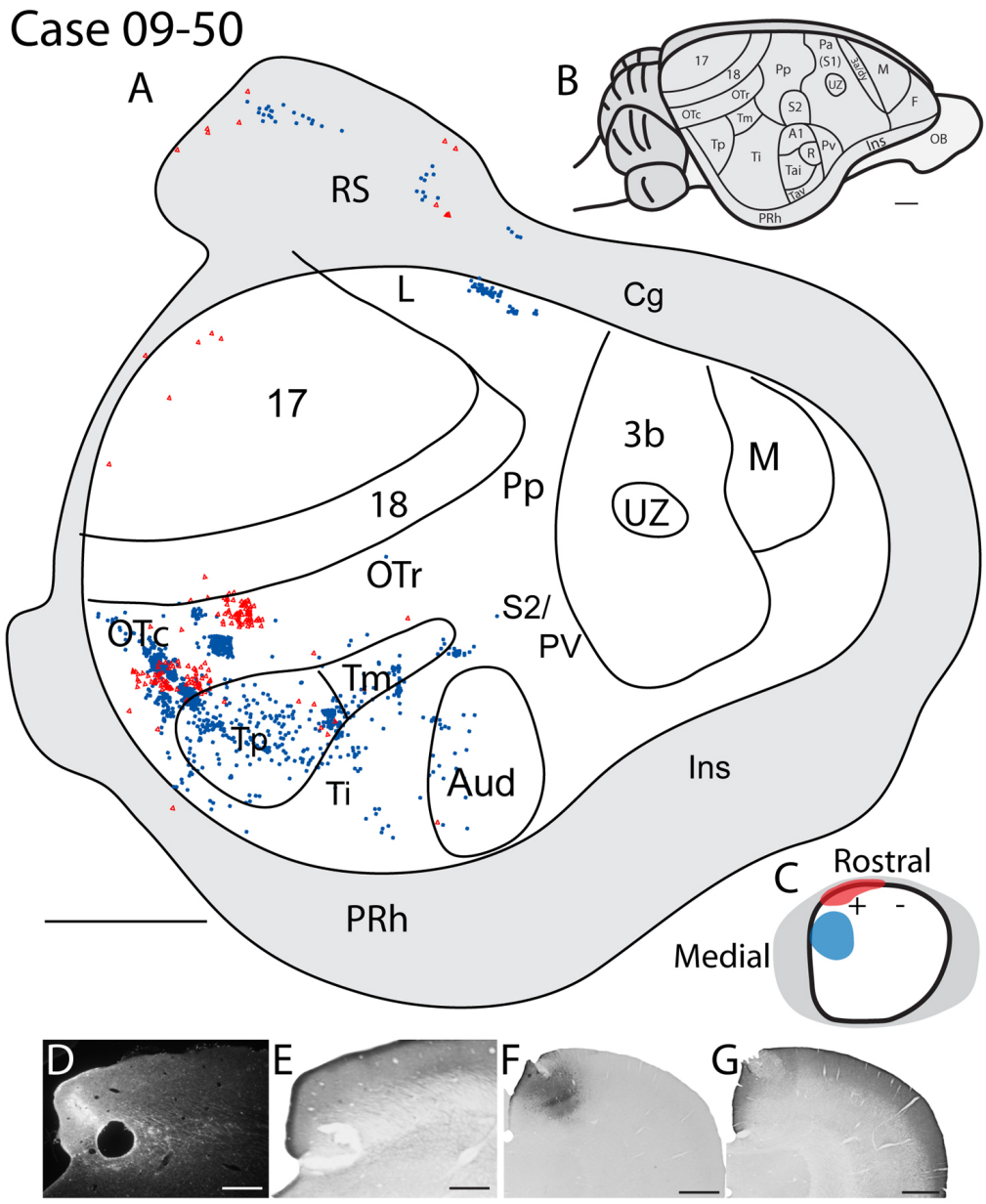


*Figure 6.4. Reconstruction of labeled cells in cortex after injections of CTB and FR into the superior colliculus of case 09-50. **A**. Shows the distribution of cortical cells projecting to the superior colliculus. Red dots represent FR labeled cells, blue dots are CTB labeled cells. The grey shaded area represents cortex that was unfolded during flattening. **B** Shows the organization of cortical areas in the gray squirrel adapted from Wong and Kaas (2008). **C** Is a dorsal view reconstruction of the injection sites within the superior colliculus. **D** and **E** are photomicrographs of the FR and CO coronal sections through the superior colliculus indicating the laminar location of the CTB injection site. **F** and **G** are photomicrographs of CTB and CO coronal sections through the superior colliculus indicating the depth of the injection sites. Scale bar for A is 5mm, D and E is 0.5mm, and F and G is 1mm.*

Surprisingly few labeled cells were present within area 18 for any of the cases. A few CTB labeled cells were scattered within the lateral and caudal extent of area 18 in case 11-37, as were a few FR labeled cells for case 11-42; however, no labeled cells were observed for case 09-50. The topography of area 18 is a mirror reversal of the topography of area 17 (Hall et al., 1971), and the location of labeled FR and CTB cells within area 18 for case 11-42 (Fig. 6.5) and case 11-37 (Fig. 6.6) reflected this type of organization. The majority of labeled FR cells in area 17 and 18 for case 11-42 (Fig. 6.5) were located close to the area 17/18; while the majority of labeled cells in area 17 and 18 for case 11-37 (Fig. 6.6) were far from the area 17/18 border, suggesting a mirror reversal across the are 17/18 border consistent with previous reports (Hall et al., 1971)

6.4.2.2. Occipital-temporal cortex

For all cases, multiple patches of labeled cells were present in the caudal half of the occipital temporal (OTc) cortical region after injections in the superior colliculus. For each injection site a patch of labeled cells was present close to the caudal lateral border of Tp (Figs. 6.4-6), with an additional patch of labeled cells for each injection site located more caudomedially (Figs. 6.4 and 6.6). A third patch of labeled cells was also present in the most caudal aspect of OTc for cases 11-37 (Fig. 6.6) and 11-42 (Fig. 6.5) but was less apparent for case 09-50 (Fig. 6.4). The pattern of label indicates that the OTc region consists of multiple viusotopically organized areas or modules. Where injections of CTB and FR tracers were located close together in the superior colliculus, the distributions of labeled cells in OT overlapped (Fig. 6.6), but where injections did not overlap within the superior colliculus, the patches of labeled cells did not overlap in cortex (Figs. 6.4 and

6.5). Consistent topographic patterns of the patches of labeled cells were difficult to ascertain in the present study from these three cases.

For all injections no labeled cells were present within the rostral occipital temporal region (OTr).

6.4.2.3. Tp, Td, and Ti

Labeled cells were observed within Tp for all injections; however, labeled cells within Tm and Ti were only present where injection sites included the lower portion of the SGI within the superior colliculus (Figs. 6.4-6). The label within Tp was often spread throughout the entire region suggesting that Tp may not be topographically organized. Few FR cells were observed within Tp for case 09-50 (Fig. 6.4), with the most superficial injection site, and for case 11-42 (Fig. 6.5), another superficial injection site. More dense patches of labeled cells were located close to the presumptive Tp/Tm border for all cases, suggesting either an error in our border demarcations, or an additional domain between Tp and Tm.

Labeled cells were only present within Tm after injections included intermediate layers of the superior colliculus (CTB injections for all cases: Figs. 6.4-6). Cells were mainly located in rostrolateral locations within Tm close to the Tp border (Figs. 6.4-6), which could reflect a topographic organization within Tm where the upper visual field is represented rostrolaterally. In case 11-42 (Fig. 6.5) multiple patches of labeled CTB cells were present within Tm, but such patches were not apparent in other cases (Figs. 6.4 and 6.6).

Within Ti, two patterns of labeled cells were observed. Close to the Tp and Tm borders cells were organized in possible patches (Figs. 6.5 and 6.6). However, in more

rostral and ventral locations cells were more distributed suggesting that there is no identifiable topography in ventral Ti.

6.4.2.4. Auditory cortex

Few labeled cells were present within auditory cortex in cases 09-50 (Fig. 6.4) or 11-42 (Fig. 6.5), but many CTB labeled cells were present within rostroventral auditory cortex in case 11-37 (Fig. 6.6), which coincided with the deepest injection site of all cases involving all layers of the superior colliculus. The distributions of cells were scattered and not patchy suggesting weak topography. Additionally, the labeled cells were not located within the primary auditory cortex, which is thought to be present in the most dorsal/medial portion of the auditory region depicted in our figures (see Fig. 6.6B for comparison) (Wong and Kaas, 2008).

6.4.2.5. Somatosensory cortex

Somatosensory cortex includes areas 3b, S2/PV, and the unresponsive zone (UZ). Labeled cells were primarily present within the S2/PV region after injections involving the intermediate layers of the superior colliculus (Figs. 6.5 and 6.6), but no cells were observed within area 3b or UZ, except for a few CTB labeled cells within the medial caudal portion of 3b for case 11-37 (Fig. 6.6). Cells in S2/PV were relatively diffusely spread out (Fig. 6.6) but had some patchy characteristics possibly indicating a weak topography (Fig. 6.5) suggesting matching topographies between the superior colliculus and divisions within S2/PV. However, the labeled cells were located more medially and therefore were likely within S2 rather than PV (Krubitzer et al., 1986).

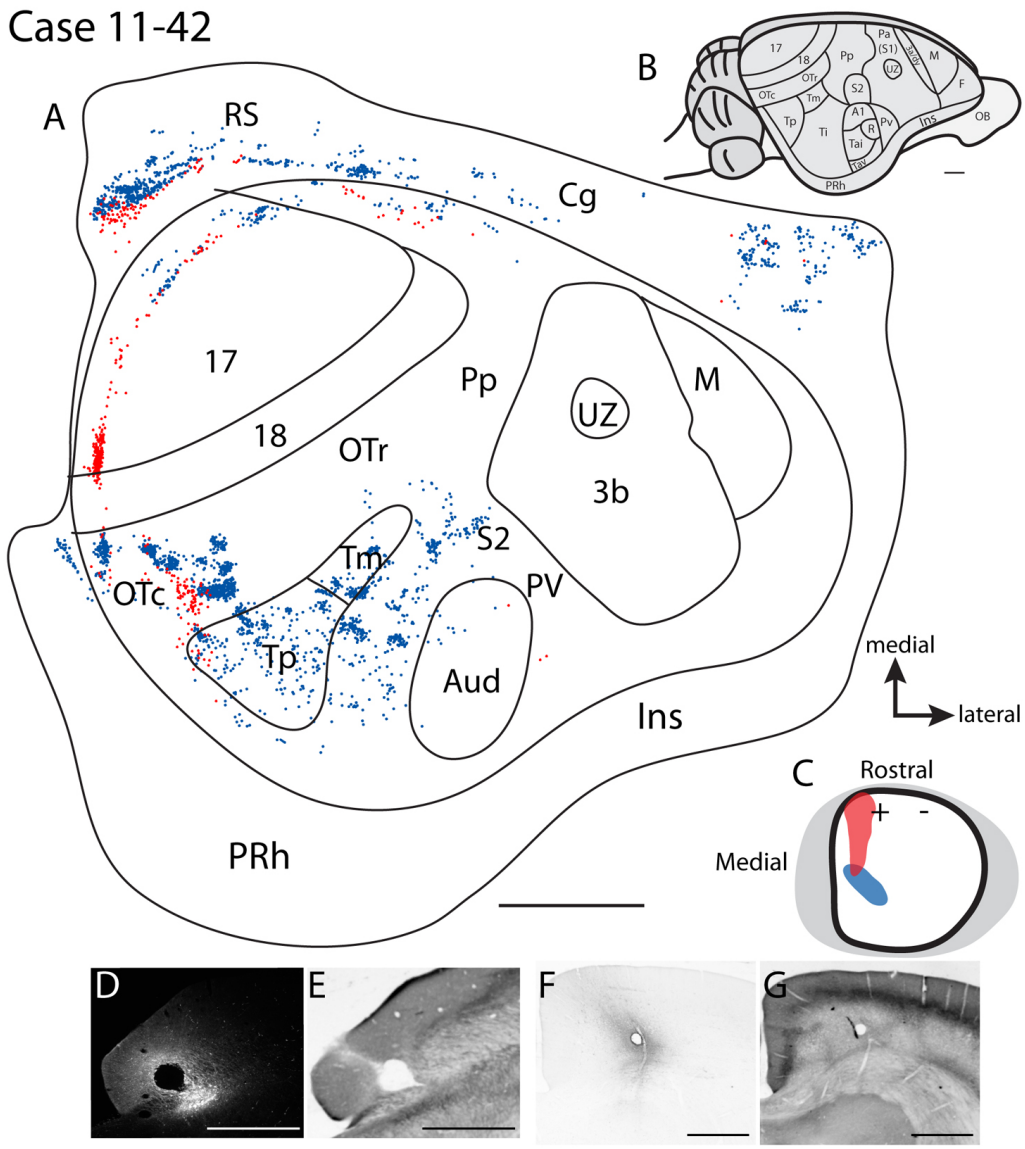
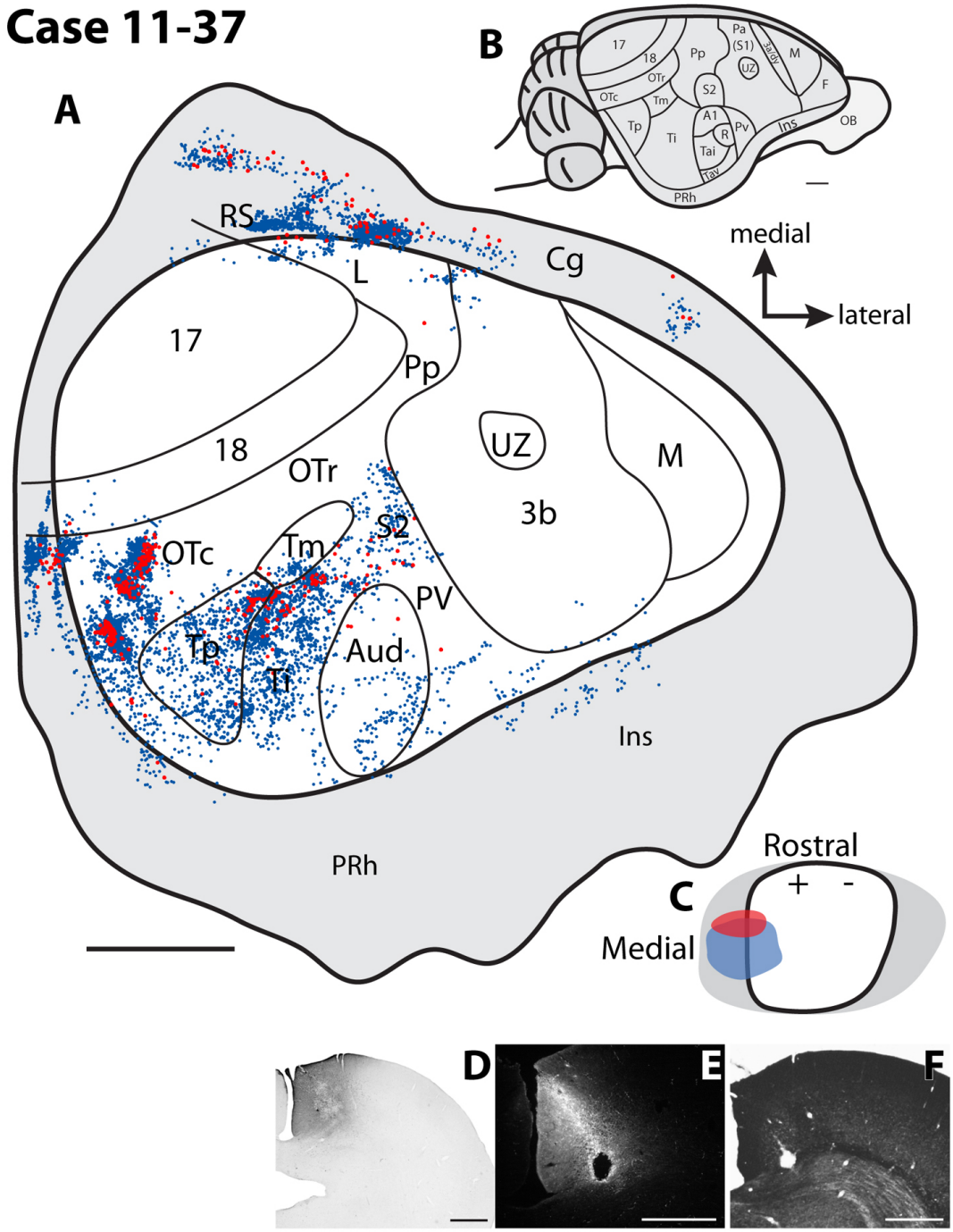


*Figure 6.5. Reconstruction of corticotectal projecting cells into the medial wall of the superior colliculus of gray squirrel case 11-42. **A** shows the location of labeled cells projecting to the superior colliculus revealed after retrograde tracers of CTB (Blue dots) and FR (red dots) were placed along the medial wall of the superior colliculus. The grey shaded area represents cortex that was unfolded during flattening. **B**. The organization of cortical areas based on Wong and Kaas (2008). **C** The location of the injection sites on a dorsal view reconstruction of the superior colliculus. **D** is a coronal section through the superior colliculus reveling the location and extent of the FR injection site. **E** is an adjacent section processed for CO to indicate the laminar location of the FR injection site. **F** Is a coronal section through the superior colliculus processed for CTB and **G** is the adjacent CO processed section used to help determine the laminar distribution of the CTB injection site. Scale bars are 5mm for A, and 1mm for D-G.*

Figure 6.6. Reconstruction of labeled cells after injections of CTB and FR into the medial wall of the superior colliculus of a gray squirrel case 11-37. Red dots represent the location of FR labeled cells while blue dots represent CTB labeled cells. Borders were determined using adjacent sections stained for myelin. The gray shaded area is cortex that was unfolded during flattening. ***B****. A small version of the organization of cortical areas within gray squirrels (adapted from Wong and Kaas, 2008) to provide a reference for possible location of labeled cells within regions.* ***C****. The location of injection sites on a dorsal view reconstruction of the superior colliculus.* ***D*** *is a photomicrograph of a coronal section through the superior colliculus processed to reveal the location of the CTB injection site.* ***E*** *is a photomicrograph of a coronal section through the superior colliculus showing the location and extend of the FR injection site.* ***F*** *is a photomicrograph of an adjacent section to E processed for acetylcholinesterase (AChE) and shows where the FR injection site is located. Scale bar for A is 5mm, D-F is 1mm.*



6.4.2.6. Retrosplenial, limbic, cingulate, and insular cortex

After superficial injections, few if any labeled cells were present within retrosplenial, limbic, cingulate, or insular cortex (Fig. 6.4). However, when injections involved deeper layers, labeled cells were present in retrosplenial, limbic, and cingulate

cortex, with our deepest injections resulting in labeled cells in insular cortex (Fig. 6.6). Within the retrosplenial cortex, the pattern of label was diffusely spread out, but hints of a topographical organization pattern suggested that central vision is located closer to area 17 and peripheral vision is located further medial (Figs. 6.5 and 6.6).

Limbic cortex contained a few scattered cells for cases 09-50 and 11-42 (Figs. 6.4 and 6.5), but a large number of cells were located within limbic cortex after our deepest superior colliculus injection of CTB (Fig. 6.6). Two patches seemed present, one more rostral than the other (Fig. 6.6) suggesting this region may be composed of two areas or modules. However, the presence of two patches was less obvious for case 11-42 (Fig. 6.5).

A few scattered cells were located in the middle rostral/caudal portion of the cingulate cortex, and an additional patch of labeled cells was present within the most rostral aspect of cingulate cortex along the medial wall of the cerebral hemisphere for cases 11-42 (Fig. 6.5) and 11-37 (Fig. 6.6). The position of these cells were rostral to the primary motor area.

In summary, most cells projecting to the superior colliculus in the three cases studied were from temporal cortical areas. Cells within occipital and temporal cortex were only observed with our most superficial injections, while deeper injections resulted in labeled cells within somatosensory, auditory, limbic, and frontal cortical regions. No cells were observed within posterior parietal cortical regions, nor were cells observed within primary somatosensory or motor cortical areas.

6.5. DISCUSSION

This report is the first to present the areal distribution of cortical projecting neurons throughout all of cortex in gray squirrels. Corticotectal projections were studied after making restricted injections of the anatomical tracers CTB and FR along the medial wall of the superior colliculus of three gray squirrels. Most superior colliculus injections included intermediate layers of the superior colliculus, with deviations in whether the tracer spread included more superficial or deeper layers. Our results support a number of conclusions which include: 1) that there are multiple areas or modules within the caudal occipital cortex, and within Ti; 2) area Tp is likely not retinotopically organized; 3) aspects of retrosplenial and limbic cortex likely have some visual functions; and 4) higher order somatosensory, auditory, and probably motor cortices project to intermediate or deep layers of the superior colliculus. In contrast to other rodents, we found few labeled cells within primary motor and somatosensory cortical areas; however, additional cases are required to definitively assess these results. Our results will be further discussed in relation to findings in other Eurachontoglire mammals, with an emphasis on describing differences and similarities between the gray squirrel and other common rodent models such as the rat and mouse, as well as with tree shrews and primates.

6.5.1. Occipital cortical areas

In the present study, we defined the occipital cortex to include areas 17 (striate cortex), area 18, the occipital-temporal region which has been split into a number of subdivisions including OTc and OTr (Kaas et al., 1989), as well as limbic and retrosplenial cortex as described in Kaas et al., 1989. Historically this region has been divided in a number of different ways (see Van Hooser and Nelson, 2006; and Krubitzer

et al., 2011 for reviews). For instance, instead of OTc and OTr, some reports have combined these areas into a single area, area 19 (Hall et al., 1971; Wong and Kaas, 2008); while others have suggested the presence of two other cortical areas in the region of OTc and OTr with very different borders such as the middle (M) and middle lateral (ML) areas (Paolini and Sereno, 1998). Therefore, the number and organization of cortical areas between areas 18 and Tp are yet uncertain, and our current results add further distinctions to those described previously.

6.5.2. Area 17 and 18

Striate cortex (area 17) in the squirrel is four times larger than that of a rat with a similar body size (Paolini and Sereno, 1998) suggesting that visual processing is more important in diurnal arboreal squirrels than rats. The superior colliculus is also proportionately large, and its role in visually guided behaviors may benefit by visual cortex having strong connections with the superior colliculus. Surprisingly though, we found very few labeled cells within area 17, and even area 18, after injections into the superior colliculus for all three cases. The small number of labeled cells in these areas could be related to the injection site depths, as the core location for all injection sites was below the lower SGS, which is known to receive projections from area 17 in squirrels (May, 2006). However, in case 09-50 (Fig. 6.4F), the CTB injection site did include aspects of the lower SGS and still very few labeled cells were observed in either area 17 or 18. The lack of labeled cells could also be explained by tissue loss during flattening, especially tissue at the most caudal aspect of area 17. The case with the most labeled cells in area 17 (11-42: Fig. 6.5) contained injection sites within the superior colliculus that were more displaced from the medial wall than the other two cases. Labeled cells for this

case (Fig. 6.5) were quite caudal within area 17, which matches descriptions of the topography of area 17 (Hall et al., 1971). We would expect labeled cells for the other two cases, 11-37 (Fig. 6.6) and 09-50 (Fig. 6.4) to be located even more caudally and medially within area 17 than the labeled cells observed for case 11-42 (Fig. 6.5) given their injection site locations within the superior colliculus.

Previous reports studying injections of anatomical tracers in area 17 resulted in terminal label within the lower SGS (Robson and Hall, 1977; May, 2006), but projections from area 18 and cortical areas lateral to area 18 resulted in terminal label mostly within the dorsal SGI (See figures 18, 19 and 20 of Robson and Hall, 1977). In our unpublished work we also found projections from area 17 to the lower SGS from tracer injections placed within area 17. However, the distribution of the terminal label covers a very small region and the amount of terminal label is weak, at least weaker than the terminal label distribution within the LGNd. We are uncertain as to why there is lack of labeled cells within area 18 for any of our cases.

Projections from area 17 and visual areas bordering area 17 to the superior colliculus have been reported in a number of Euarchontoglires species such as rats (Olavarria, 1982; Harvey and Worthington, 1990), rabbits (Holländer and Schönitzer, 1983; Müller-Paschinger and Tömböl, 1989), tree shrews (Casseday et al., 1979; Huerta et al., 1985; Chapter 5 of current report), and primates (Fries, 1984; Cusick, 1988; Lock et al., 2003; Collins et al., 2005; Baldwin and Kaas, 2012), suggesting this type of projection is a common feature among the Euarchontoglires clade.

In primates, area 17, or V1, sends dense projections to the superior colliculus (Fries, 1984; Lock et al., 2003; Collins et al., 2005; Baldwin and Kaas, 2012), and in fact,

the highest number of cells that project to the superior colliculus in primates arise from V1 (Collins et al., 2005; Baldwin and Kaas, 2012). Yet, in the current study of squirrels, even when area 17 did have labeled cells, projections from other cortical areas within OTC and the retrosplenial cortex were just as dense (Fig 6.5).

V1 receives most of its incoming visual information from the lateral geniculate nucleus and receives little or no input from the extrageniculate pathway, which passes visual information through the superior colliculus and pulvinar (Robson and Hall, 1977). The weak corticotectal input observed in the present cases and our unpublished data could reflect differences in functional streams associated with the striate and extrastriate pathways. In other words, cortical regions that receive inputs from the LGN and not the superior colliculus/puvlinar may not provide much feedback to the superior colliculus, while the extrastriate visual areas, which do receive input from the extrageniculate pathway provide feedback.

6.5.3. OTc and OTr

OTc and OTr, which are divisions of the occipitotemporal cortex, are located lateral to area 17 (Kaas et al., 1989) (Fig. 6.1). For all cases in the present study, the strongest corticotectal projections were from the occipital-temporal region, specifically the caudal occipital temporal region. Multiple patches of label were observed within OTc suggesting this region is likely composed of multiple areas or modules. Additionally, when injections within the superior colliculus were separate and non-overlapping the resultant cortical label was also non-overlapping providing evidence for the presence of visuotopically organized fields.

Given the overlap between M and ML domains described in ground squirrels by Paolini and Sereno (1998) and the location of OTc and OTr in gray squirrels (Kaas et al., 1989; Krubitzer et al., 2011), it is likely that this region of cortex projects to the superior colliculus and contains cells that are direction selective and have a topographic organization. Also, ML and M are topographically organized, as the projection patterns within our cases suggest. However, the borders of M and ML of Paolini and Sereno (1998) are not consistent with our current report, but M and ML are positioned between the borders of area 18 and Tp.

In a number of studies, the OT region has been given the name of area 19 (Kaas et al., 1972; Wong and Kaas, 2008); however, that term seems inappropriate as homologies in cortex lateral to area 18 are uncertain. What does seem clear is that OT is made up of several subdivisions. Differences in connections between regions of OT are observed in callosal connections (Kaas et al., 1989). There are also differences in corticopulvinar connections between different locations within the OT region (Robson and Hall, 1977). In the present study, further evidence suggests that the OT region has several divisions, and possibly several subdivisions within OTc. We observed few, if any, cells within the rostral aspect of OT; therefore, it is likely that OT is not a single visual field like area 19 (area V3) in some other mammals such as cats and primates.

In tree shrews, evidence for multiple cortical areas along the rostral border of area 18 has been reported in a number of studies (Sesma et al., 1984; Lyon et al., 1998: also see Chapter 5 of current report); and, likely multiple fields are present within the most caudal aspect of the region rostral to area 18, as evidenced by multiple patches of tectal projecting cells within the caudal occipital-temporal region (Chapter 5 of current report).

Therefore, it is likely that area 19/V3 was not a common visual area shared by all Euarchontoglires, but emerged with the evolution of primates.

6.5.4. Limbic and retrosplenial cortex

Area 17 is bordered medially by retrosplenial cortex, and rostromedially by the presumptive limbic cortex (Kaas et al., 1972) (Fig. 6.3). Strong projections to the superior colliculus arise from both the limbic and retrosplenial cortical regions, found especially after injections into the intermediate and deep layers of the superior colliculus (Figs. 6.5-6). Within the limbic region there appears to be two large clusters of cells (Fig. 6.6), while the distributions of cells within the retrosplenial cortex are more diffusely distributed (Figs. 6.5-6). The limbic region is known to receive projections from area 18 (Robson and Hall, 1977; Kaas et al., 1989), as well as area 17 (unpublished data); however, limbic cortex has not been found to be responsive to visual stimuli in electrophysiological recording experiments of anesthetized squirrels (Hall et al., 1971).

6.5.5. Temporal cortical areas

The relatively large temporal cortex of gray squirrels consists of three main regions, the temporal intermediate region (Ti) and a more caudal region, which includes the temporal posterior (Tp) and temporal medial fields (Tm) (Wong et al., 2008; Wong and Kaas, 2008).

6.5.6. Temporal intermediate region

The Ti region includes the region of cortical tissue that stains poorly for myelin rostral to Tp and Tm, but caudal to auditory cortex (Fig. 6.1 and 6.3) (Kaas et al., 1989; Wong and Kaas, 2008). Projections from this area to the superior colliculus are observed

when injections include intermediate and deep layers of the superior colliculus (Figs. 6.5 and 6.6), with the distribution of labeled cells somewhat patchy more medially and diffuse more laterally. This pattern suggests that Ti may have multiple subdivisions with the more medial half containing one or more areas that are topographically or modularly organized and the lateral half having a poor or crude topographic organization. Ti receives little or no projections from areas 17 and 18 (Kaas et al., 1989) and only sparse inputs from auditory cortex (Luethke et al., 1988), but Ti does receive projections from subcortical auditory and multisensory centers such as the medial geniculate complex and the suprageniculate nucleus (Wong et al., 2008). Thus, Ti has been suggested to be similar to the auditory area Te2 of rats (Wong et al., 2008) because of similarities in cytoarchitecture and connections (Arnault and Roger, 1990; Clerici and Coleman, 1990). Ti is in a similar position to a region with known projections to the superior colliculus in tree shrews that originate just caudal to auditory cortex within the inferior temporal cortex (See Chapter 5 of current report). However, very little is known about connections between Te2 or other proposed homologous structures with the superior colliculus in other mammals.

6.5.7. Temporal posterior area

The temporal posterior area, Tp, is well defined by its dark myelination relative to surrounding cortex (Fig. 6.3) (Kaas et al., 1998; Wong and Kaas, 2008). All cases with CTB injections in the current study resulted in labeled cells within Tp, however, few FR cells were observed within Tp. We believe this difference reflects differences in tracer sensitivity as the depths of FR injection sites were similar to the depths of CTB injection sites across cases. The distribution of labeled cells within Tp was diffusely spread out

throughout the entire area defined by myelin staining suggesting that Tp has a relatively poor topographic organization pattern. This observation is consistent with studies of neuronal properties in area Tp indicating that cells in Tp have large receptive fields and no apparent topographical organization pattern (Hall et al., 1971).

Tp receives dense inputs from the caudal subdivision of the pulvinar complex (Robson and Hall, 1977; Wong et al., 2008), which appears to only have a crude visuotopic organization (Robson and Hall, 1977; Baldwin et al, 2011). Therefore Tp receives inputs from the superior colliculus through the pulvinar and provides possible feedback information to that pathway. Other inputs are from the medial and dorsal divisions of the medial geniculate complex (Wong et al., 2008), suggesting that Tp could be involved in both visual and auditory processing. Yet few, if any, connections are observed between Tp and auditory cortex (Luethke et al., 1988). Tp also shares few to no connections with area 17 (Kaas et al., 1989; Cusick et al., 1980) and receives only minor projections from area 18 (see Kaas et al., 1989).

The temporal posterior cortex described in tree shrews appears to be different from that described in gray squirrels. Both the position of these areas are different and their connections differ as well, with Tp in squirrels displaced from the area 18 border (Fig. 6.3), unlike Tp as described in tree shrews (Wong and Kaas, 2009, also see Chapter 5 of current report). Yet the lateral aspect of IT described in tree shrews, which is in a similar location as Tp in squirrels, also exhibits a diffuse pattern of labeled cells after retrograde tracers are injected into the superior colliculus (Chapter 5 of current report).

6.5.8. Temporal medial area

Tm stains darkly for myelin, but less darkly than Tp (Wong and Kaas, 2008), and receives inputs from the pulvinar, but not from the medial geniculate nucleus (Wong et al., 2008). The projections from the pulvinar are likely from the rostral lateral pulvinar, which receives topographically organized projections from the superior colliculus. Additionally, Tm may receive projections from area 18 (Kaas et al., 1989). These connectional characteristics suggest that Tm is visual in function. The projections that Tm sends to the superior colliculus seem to be topographically organized, as the pattern of labeled cells within Tm are tightly clustered in patches and not diffusely distributed. The location of Tm is in a similar location to ITc in tree shrews, or to the location between ITi and Ti, which also may be topographically organized (See Chapter 5 of current report). But, we are uncertain regarding homologous structures to Tm in tree shrews or other mammals.

6.5.9. Somatosensory cortex

In the present study we define somatosensory cortex as including areas 3b, UZ, S2, and PV. Labeled cells were present within the S2/PV region, and likely were localized more within S2 than PV (see Krubitzer et al., 1986). We did not observe projections to the superior colliculus from primary somatosensory cortex (3b) or the unresponsive zone. Previous reports have suggested that somatosensory inputs from the unresponsive zone to the intermediate and deep layers of the superior colliculus are present (Gould et al., 1989). The lack of labeled cells in somatosensory areas in the present study could be a result of our tracer placement in that our injections did not include layers of the superior colliculus that receive projections from somatosensory

areas. However, Gould et al. (1989) found that terminal label was located as ventral within the superior colliculus as the SGI after cortical injections of anterograde tracer within the unresponsive zone. Most of our terminal label was located within the lateral superior colliculus, a region of the superior colliculus not included in our present report. Yet, it is unlikely that the unresponsive zone shares connections with only the lateral superior colliculus and not medial aspects. However, differences between medial and lateral superior colliculus connections with both cortex and subcortical structures have been reported (Dean et al., 1986; Sahibzada et al., 1986; Comoli et al., 2010; Favaro et al., 2011; Comoli et al, 2012), yet these differences could also reflect difference in the topography of the projecting areas.

In other rodent species, projections to the superior colliculus from primary and secondary somatosensory areas have been reported (mouse: Aronoff et al., 2010; rat: Wise and Jones, 1977; Harvey and Worthington, 1984; Hoffer et al., 2005; Comoli et al., 2012 hamster: Rhodes et al., 1981). In primates, projections from higher order somatosensory areas such as S2/PV have been reported (Fries et al., 1984; Collins et al., 2005; Wu et al., 2005; Baldwin and Kaas, 2012), but superior colliculus connections with primary somatosensory cortex for either primates or tree shrews have not been observed (Fries et al., 1984; Collins et al., 2005; Baldwin et al., 2005; Chapter 5 in current report). These differences in somatosensory influences on the superior colliculus between some species of the Euarchontoglire clade could be a result of differences in behavioral characteristics more suitable for arboreal versus ground dwelling habits, and the use of navigating environments visually over using somatosensory inputs from whiskers.

6.5.10. Motor and Cingulate cortex

The motor cortex of squirrels is less myelinated than, and located more rostral to, somatosensory cortex (Wong and Kaas, 2008; Cooke et al., 2011). In the present report we did not observe any labeled cells within motor cortex. Again, the reason for this lack of label could be a result of our superior colliculus injections only including medial aspects of the superior colliculus (see discussion above). Projections from primary motor areas in primates have not been reported; however, such projections have been described in rats (Miyashita and Mori, 1995; Alloway et al., 2010; Comoli et al, 2012).

The cingulate cortex is located along the rostral half of the medial wall in squirrels and has been divided differently based on architecture (see Wong and Kaas, 2008 for review). The caudal portion of the cingulate cortex is thought to be involved with limbic processes, with the rostral portion involved in motor functions. Labeled cells were primarily located in the rostral portion of cingulate cortex only after injections into the superior colliculus involving deep layers of the superior colliculus (Figs. 6.4-6). Therefore, it is likely that this region may be involved in motor influences upon the deep layers of the superior colliculus, as the deeper layers of the superior colliculus are associated with motor functions (see May, 2006 for review).

May (2006) showed similar patterns of connections between the superior colliculus and this region of cortex, and such patterns have also been observed in rats (Beckstead, 1979). Both of these authors, as well as other studies in rodents within this region (Hall and Lindhom, 1974; Donahue and Wise, 1982; Neafsey et al., 1986; Rapisarda, 1990; Stuesse and Newman, 1990; Tsumori, 2001) suggest that this corticotectal projecting region could be equivalent to the frontal eye fields of primates,

but further experiments aimed at elucidating the functional characteristics of this region of cortex in squirrels need to be conducted.

6.5.11. Auditory cortex

The auditory cortex described in this chapter has been suggested to be composed of the primary auditory cortex (Merzenich et al., 1976), located along the more rostral aspect, and multiple other auditory areas (Luethke et al., 1985), in more lateral and rostral locations. Labeled cells were observed within the auditory cortex (Fig. 6.6), but the majority of those cells were likely outside of the primary auditory cortex. This result is similar to observations in tree shrews (Casseday et al., 1979; Chapter 5 of current report) and primates (Fries et al., 1984; Collins et al., 2005; Baldwin and Kaas, 2012).

6.5.12. Squirrels and other rodents

This section of the discussion will focus on general differences between squirrels and other commonly studied rodents and how those differences may be reflected in corticotectal projections as well as the overall structural differences within the superior colliculus. Rodents are popular animal models for studying brain and neuronal functions including studies of the visual system. Though the range of the rodent order is vast, including 34 families and up to 2277 species, the majority of studies on rodents are conducted using rats and mice (Manger et al., 2008; Krubitzer et al., 2011). Substantial differences in behavioral repertoire are obvious between squirrels and the two popular rodent models of rats and mice. For one, squirrels are diurnal whereas the majority of rats and mice are nocturnal. Additionally, squirrels use vision to navigate throughout the environment, while rats and mice rely heavily on their vibrissae. Such behavioral differences are reflected in the cortical organization of these different species. For

instance, the striate cortex of squirrels is four times larger and extrastriate cortical areas are 8 times larger than those found in rats with similar body sizes; conversely, the barrel fields of rats are three times the size of those observed in squirrels (Paolini and Sereno, 1998). Considering that one of the key roles of the superior colliculus is to integrate sensory inputs to produce orienting movements, it seems likely that such cortical differences in behavior are also reflected in the corticotectal inputs.

In summary, the majority of cortical projections to the superior colliculus arise from visual cortical areas, with additional projection coming from non-primary auditory and non-primary somatosensory cortical areas. Additionally, projections arise from the anterior cingulate, insular cortex, retrosplenial, and limbic cortex. This general pattern of cortical inputs to the superior colliculus is somewhat of a hybrid between the projection patterns observed in other popular rodent models such as rats and mice, as well as primates. Some of the differences in corticotectal projections observed in rats and mice versus squirrels were the lack of cortical projections from primary somatosensory and motor cortex, especially from regions of cortex associated with barrel fields. The major differences between primates and the gray squirrel were the lack of projections from posterior parietal cortical areas and the presence of cortical projections from retrosplenial and limbic cortex. Differences in the corticotectal projections across these species are likely attributable to differences in behavioral characteristics that are also reflected in cortical organization differences.

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CHAPTER 7

SUMMARY AND CONCLUSIONS

The current research is part of a larger effort to understand how the human brain evolved. These particular studies address questions about the evolution of the visual system in early primates such as galagos (*Otolemur garnettii*), and mammals closely related to primates such as tree shrews (*Tupaia* glis) and gray squirrels (*Sciurus carolinensis*). These species were chosen for several important reasons. All three species are highly visual mammals with well-developed visual systems, share similar ecological environments, and are all members of the same phylogenetic mammalian radiation, the Euarchontoglires. Therefore, these species likely share many common brain features, but also have divergent specializations reflecting differences in their evolutionary history. An emphasis was placed on understanding the connections of the visual brain structures with the superior colliculus, a key structure within the extrageniculate pathway, which is also involved in producing orienting movements. Injections of anatomical tracers were placed within the superior colliculus of all three species, while various histochemical and immunohistochemical procedures were used to reveal architectonic characteristics of the superior colliculus, pulvinar, and cortical visual areas. Subcortical connections were analyzed in galagos and gray squirrels (Chapters 2 and 3), while cortical connections were analyzed in all three species (Chapters 4, 5 and 6). What follows is a summary of the conclusions grouped together by their main aim, which include: 1) Determining the subcortical connections of the superior colliculus in gray squirrels and galagos, with the goal of gaining insights into the organization of the visual pulvinar, as well as assessing

possible homologous structures within the pulvinar complex between rodents and primates (covered in Chapters 2 and 3); and 2) Understanding the corticotectal projections in gray squirrels, tree shrews, and galagos, and gaining insights into how modifications of cortex, such as the expansion of temporal visual and parietal visuomotor regions, are reflected in tectal inputs (covered in Chapters 4, 5 and 6).

7.1. AIM 1: SUBCORTICAL CONNECTIONS OF THE SUPERIOR COLLICULUS

In order to study the subcortical projections of the superior colliculus in gray squirrels and galagos, we placed anatomical tracers within the superior colliculus at various retinotopic locations and analyzed other subcortical visual structures for either anterograde terminal label or retrogradely labeled cells. One of the major projections of the superior colliculus is to the pulvinar complex, an important visual structure in the thalamus (Purushonthaman et al., 2012), and a source of visual input for much of extrastriate cortex (see Jones, 2007 for review). Therefore, much of chapters 2 and 3 were focused on revealing the organization of the pulvinar complex. The staining procedures, in conjunction with our analysis of anatomical connections between the superior colliculus and pulvinar complex in galagos and gray squirrels, allowed us to reveal new subdivisions within the pulvinar complex of each species, as well as determine possible homologous subdivisions between rodents and primates.

In chapter 2 (Baldwin et al., 2011), we studied the tectal projections to the pulvinar nucleus in gray squirrels and discovered that the pulvinar complex of squirrels consists of four subdivisions, the caudal pulvinar (C), the rostral medial pulvinar (RM), and two divisions of the rostral lateral pulvinar (RL), one being lateral (RLl) and the other medial (RLm). Projections from the superior colliculus to the divisions of the rostral lateral pulvinar were topographically organized, while the projections to the caudal pulvinar were more diffuse. There was an additional subdivision, RM, of the gray squirrel pulvinar that did not receive any projections from the superior colliculus. Furthermore, these divisions could be differentiated from one another based on histological staining procedures with the most notable characteristics being that the

caudal pulvinar stains darkly for AChE, CO, and VGLUT2. When comparing our results with studies of cortical connections of the pulvinar (Kaas et al., 1972; Robson and Hall, 1977; Wong et al., 2008) we concluded that the caudal pulvinar shares connections with visual temporal areas while the two subdivisions within RL share connections with occipital temporal areas, including the temporal medial area, Tm (Kaas et al., 1972; Robson and Hall, 1977; Wong et al., 2008). This suggests that there are likely two pathways through the extrageniculate pathway leading from the retina to cortex. Since the time of our study, additional evidence of two pathways through the superior colliculus to the pulvinar complex have been proposed with the pathway that travels through the caudal division processing motion stimuli (Fredes et al., 2011). These two pathways seem to originate from two different layers of the superior colliculus, with the caudal division pathway originating in the lower SGS, and the rostral divisions pathway originating within the stratum opticum (Fredes et al., 2011)

In chapter 3, we studied the tectal projections of the pulvinar nucleus in prosimian galagos. This study showed that the superior colliculus projects to two locations within the caudal pole of the pulvinar complex, which we named the posterior pulvinar (Pp), and the posterior central pulvinar (Ppc). Additional projections from the superior colliculus were to the lateral pulvinar (PL), as well as to more medial aspects likely within subdivisions of the inferior pulvinar (PI). There were no tectal projections to the medial pulvinar (PM). One of the more exciting aspects of this study was that the two caudal divisions of the pulvinar in galagos could be discerned by their dark VGLUT2 staining and, in ideal cases, dark CO staining. When comparing our results with studies of cortical connections with the pulvinar, we found that the posterior pulvinar shares connections

with temporal visual structures, while connections to more lateral and rostral divisions of the pulvinar share connections with occipital visual structures (Glendenning et al., 1975; Raczkowski and Diamond, 1981; Wong et al., 2009). Galagos have an expanded temporal visual cortex relative to gray squirrels (Wong et al., 2009; Wong et al., 2010), and the presence of two caudal/posterior divisions in the galago, versus one caudal division in the gray squirrel, that stain darkly for VGLUT2 could reflect this expansion.

Whether the projections to the more posterior divisions of the pulvinar and the more rostrolateral divisions originate from different layers or different types of cells within the superior colliculus of galagos is still uncertain. However, the distribution of VGLUT2 mRNA labeled neurons in the lower SGS of the superior colliculus (Balaram et al., 2011) provides strong evidence that this is the sublayer that projects to posterior divisions of the pulvinar in galagos. Studying this question may provide further information on the evolution of the visual pulvinar, the superior colliculus, and the extrageniculate visual pathway.

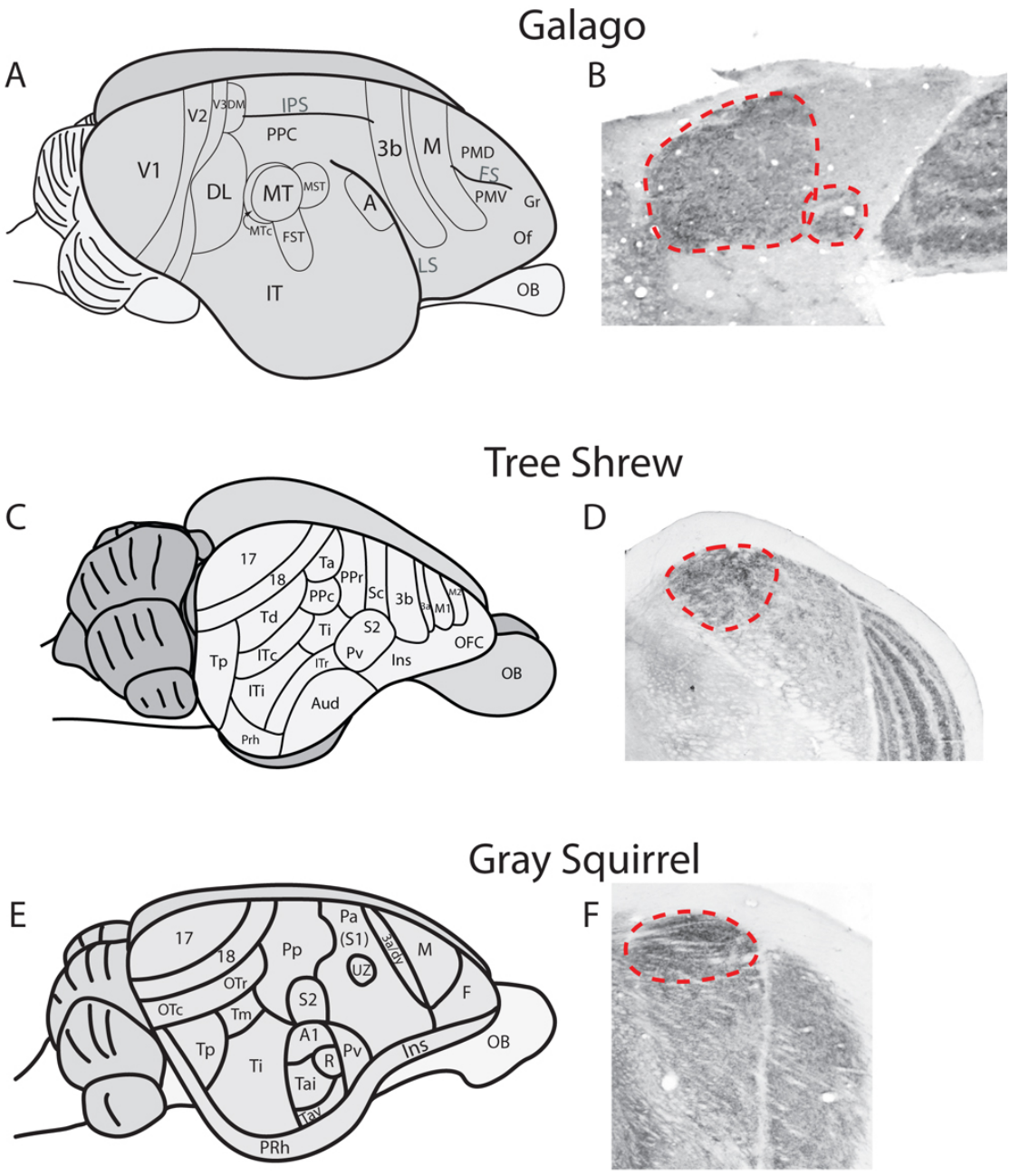

Figure 7. 1. Cortical organization of galagos (A), tree shrews (C), and gray squirrels (E) in comparison to the organization of the pulvinar complex in the same species (B, D, and F). Sections B, D, and F are coronal sections taken from a more caudal end of the pulvinar complex for each species stained for VGLUT2 protein. Note, with an expanded temporal lobe in galagos, there are additional VGLUT2 staining divisions of the pulvinar in galagos.

7.2. AIM 2: CORTICAL PROJECTIONS TO THE SUPERIOR COLLICULUS

The superior colliculus is present in all vertebrates, but the specific functions of this structure may vary depending on differences in connections. Therefore, the cortex can influence the functional properties of the superior colliculus through its corticotectal projections, which in turn project to motor centers and the visual thalamus. To assess these cortical influences, similar methodological procedures were used to identify cortical visual and visuomotor areas projecting to both superficial and deep layers within the superior colliculus of galagos, tree shrews and squirrels (Chapters 4, 5, and 6). Together, these studies helped us address whether and how the emergence of an expanded temporal, parietal, and frontal lobe is incorporated within the superior colliculus of primates, as well as determine what corticotectal projections are common to all three species.

Common corticotectal features observed in galagos, tree shrews, and gray squirrels were that injections involving superficial layers of the superior colliculus resulted in retrogradely labeled cells within early visual cortical structures; while deeper injections resulted in labeled cells in frontal cortical areas that may be involved with visuomotor functions (Fig. 7.2). Differences between the three species reflected differences in the number of cortical areas projecting to the superior colliculus and likely reflecting the expansion of cortex in primates and the incorporation of these derived regions in the superior colliculus function. What follows is a summary with greater details of the corticotectal projection pattern for each species and how they compare with each other.

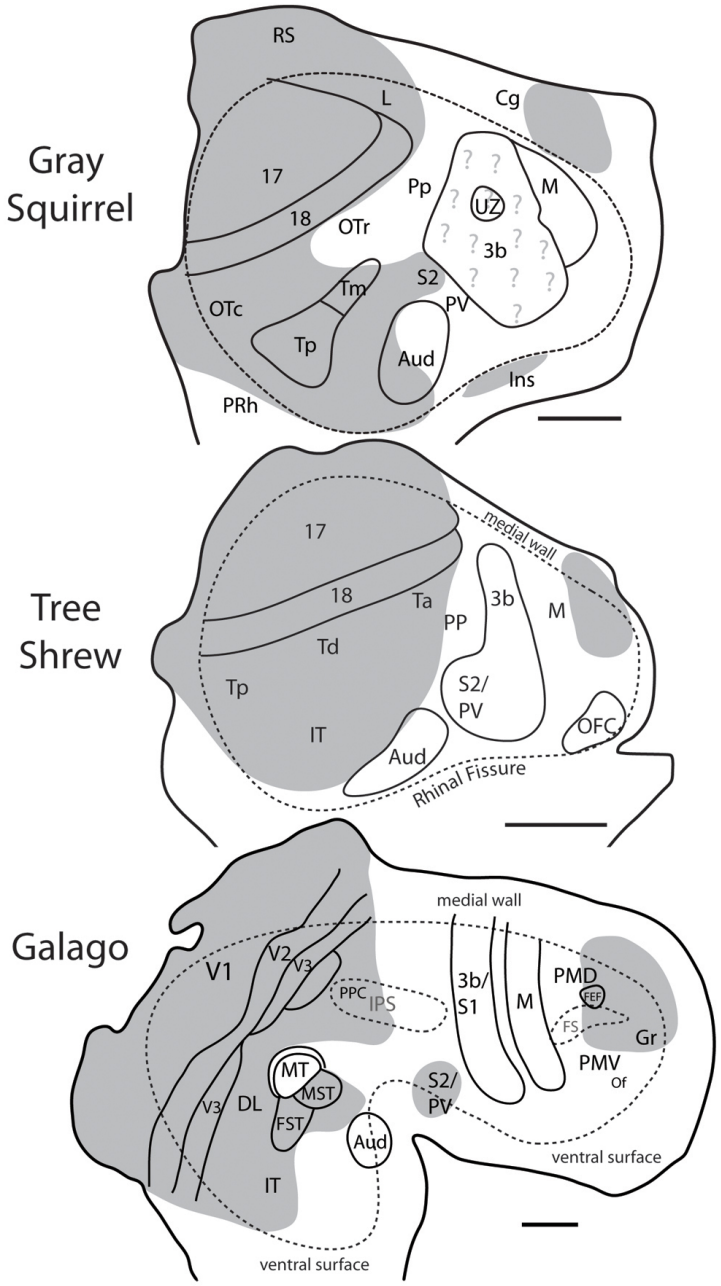

Figure 7. 2. Generalized summary of cortical projections to the superior colliculus in gray squirrels, tree shrews, and galagos. Grey shaded regions represent the locations of observed cortical projecting cells. The summaries are presented on flattened representations of the cortical surface. Medial is up, rostral is to the right. Scale bars are 5mm

In chapter 4 (Baldwin and Kaas, 2012) we studied the corticotectal projections in galagos. The majority of projections to the superficial layers were from early visual areas,

V1 and V2. A surprising result from this study was the lack of corticotectal projections to area MT, a visual area that emerged with primates (Kaas, 2002, 2003), and also has strong superior colliculus projections in other anthropoid primates (Graham et al., 1979; Fries, 1984; Lock et al., 2003; Collins et al., 2005). Instead, other MT complex areas such as FST and MST project to the superior colliculus suggesting that MT in galagos is not as involved in coordinating orienting movements as it may be in anthropoid primates. It is unclear why MT does not appear to project to the superior colliculus of galagos. However, MT may have an evolving role in vision across primates and therefore projections from MT to the superior colliculus may not be present in all primates. Additionally, we found relatively few projections from the frontal eye fields (FEF) to the superior colliculus, but we did find strong projections from surrounding cortical areas involved with other orienting movements (Chapter 4). Extensive projections to the superior colliculus from the caudal posterior parietal region were observed. The extent of projections from this region in galagos is much larger than what is observed in tree shrews (Chapter 5 of current report) and gray squirrels, who may not have corticotectal projections from the posterior parietal cortex (Chapter 6 of current report), but is similar to reports of anthropoid primates (Fries et al., 1984; Collins et al., 2005; Lock et al., 2003). The organization of the region of posterior parietal cortex that projects to the superior colliculus is not well understood in galagos; however, the most rostral aspect of the tectal projection zone may overlap with areas associated with facial movements including eye lid closure (Stepniewska et al., 2005; 2009a). Few projections originated from primary somatosensory or motor areas.

In chapter 5, the cortical projections to the superior colliculus were studied in tree shrews. Substantial projections were observed to the superior colliculus from temporal cortical areas outside of area 18. Some temporal areas had more focused and likely visuotopic organization patterns, while other temporal areas, located more rostrolaterally, likely are not topographically organized. The general locations of the topographical and crudely organized regions are similar to those observed in galagos, where labeled cells in inferior temporal cortex were scattered. A few labeled cells were observed within the region of Ta and the posterior parietal cortex after injections involving deep injections into the superior colliculus, but the extent of such projections is relatively small when comparing results between galagos and gray squirrels. Like primates, few projections originated from primary somatosensory or motor cortical areas.

Finally in chapter 6, cortical projections to the superior colliculus were analyzed in gray squirrels, a highly visual rodent. We were only able to assess the corticotectal projection patterns after injections into the medial wall of the superior colliculus, and therefore projections to more lateral aspect of the superior colliculus are not described. Therefore, our results may not include descriptions of all corticotectal projecting cells. Other reports in rats have suggested differences in cortical projections from the medial or lateral aspects of the superior colliculus (Dean et al., 1986; Sahibzada et al., 1986; Favaro et al., 2011; Comoli et al, 2012), but the results interpreted as differences in the areas projecting to medial or lateral portions of the superior colliculus may instead reflect the different topographic regions of the projections. Regardless, it would be informative to determine if such differences exist in squirrels. We did not observe projections to the superior colliculus from primary somatosensory cortex, but such reports have been

reported previously (Gould et al., 1989). Regardless, injections in more central and lateral aspects of the superior colliculus would be helpful for the present report and for a full understanding of the distribution of corticotectal projections in gray squirrels.

A major difference between the corticotectal projections in gray squirrels to those of tree shrews and primates is the lack of projections from posterior parietal cortex. However, similar patterns of labeled cells were observed in the temporal cortex of gray squirrels with respect to those observed in tree shrews and galagos where cortical areas located in caudal temporal cortex seemed to be topographically organized while more rostrolateral temporal cortex were not.

In summary, differences in cortical organization, through the expansion of temporal, parietal, and frontal cortex, are reflected by differences in corticotectal projections. However, the emergence of some brain structures are not always incorporated into the possible functions of the superior colliculus, as is the case of MT in galagos. Knowing the difference in the cortical projections across a number of mammals can help provide information as to what cortical structures are important for processes carried out by the superior colliculus, as well as provide insights into common features, and possibly common superior colliculus functions, across all mammals.

7.2.1. General concluding statement

Overall, as cortical areas evolve and change, so too do the cortical projections to the superior colliculus and the organization and complexity of the pulvinar.

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