Neurochemical Organization of Chimpanzee Inferior Pulvinar Complex

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ABSTRACT
The pulvinar of primates, which connects with all visual areas, has been implicated in visual attention and in control of eye movements. Recently, five separate neurochemical subdivisions of a region termed the inferior pulvinar complex have been identified in monkeys (Gray et al. [1999] J Comp Neurol 409:452–468; Gutierrez et al. [1995] J Comp Neurol 363:545–562), and comparable subdivisions have been mapped in humans (Cola et al. [1999] NeuroReport 10:3733–3738). In the present study, we investigated the inferior pulvinar of the chimpanzee (Pan troglodytes), the closest evolutionary relative of humans, using cytochrome oxidase (CO) and acetylcholinesterase (AChE) histochemistry, and immunocytochemistry for calbindin. Each staining method demarcated five histochemical zones corresponding, from medial to lateral, to the posterior (PIP), medial (PIM), central (PIC), lateral (PIL), and the lateral-shell (PIL-S) divisions in monkeys. The PIP division stained darkly for calbindin and lightly for CO and AChE. The PIM division was characterized by less neuropil staining for calbindin, and by distinct, intensely stained patches of CO and AChE. PIC appeared lighter than adjacent divisions with CO and AChE histochemistry and was moderately stained with calbindin. PIL was moderately to darkly stained with each method and was adjoined by a lighter staining shell, PIL-S. Thus, in the aspects of organization we examined, the inferior pulvinar of chimpanzees closely resembles that of humans and monkeys. This investigation provides a foundation for more detailed studies of the thalamic relationships of extrastriate cortex in apes and humans. J. Comp. Neurol. 484:299–312, 2005. © 2005 Wiley-Liss, Inc.

Indexing terms: visual system; thalamus; primate; evolution

Research in primate neurobiology has traditionally focused on the study of New World and Old World monkeys. By contrast, there have been relatively few studies of the ape–human (hominoid) branch of primate evolution, which separated from the Old World monkey branch ~25 million years ago (Fleagle, 1999). Recently, however, there has been renewed interest in understanding how humans both differ from and resemble other primates, and this has fueled new investigations of chimpanzees (Pan troglodytes and P. paniscus), the great ape species most closely related to humans. New studies have been published comparing humans, apes, and monkeys with respect to neuroanatomy (Preuss et al., 1999; Hackett et al., 2001; Hopkins and Pilcher, 2001; Semendeferi et al., 2001; Preuss and Coleman, 2002), gene expression in the brain (Enard et al., 2002; Cáceres et al., 2003; Clark et al., 2003; Preuss et al., 2004; Uddin et al., 2004), and cognition...
(Cheney and Seyfarth, 1990; Povinelli and Preuss, 1995; Povinelli, 2000). These studies make it clear that although humans share many features of brain organization and function with apes and monkeys, there are important differences as well. Documenting the patterns of similarities and differences is essential for understanding how results derived from nonhuman primates pertain to humans and how evolution modified ancestral primate organization to shape human neural and cognitive specializations.

Like other brain systems, the visual systems of apes and humans resemble those of nonhuman primates in some ways, but differ in others (reviewed by Tootell et al., 2003; Preuss, 2004). For example, humans exhibit features of primary visual cortex anatomy which have been posited to represent modifications of the magnocellular (M) pathway not found in apes or monkeys (Preuss et al., 1999; Preuss and Coleman, 2002). In addition, there are differences in the activation patterns of extrastriate and intraparietal areas between humans and macaque monkeys when subjects view certain classes of moving stimuli, with humans exhibiting substantial motion-related activity in several areas that are relatively insensitive to motion in macaques (Orban et al., 2003; Tootell et al., 1997; Vanduffel et al., 2002). Many aspects of visual system anatomy and function have not been subjected to detailed comparative study in primates, however. The anatomy and histochemistry of the pulvinar nucleus of the thalamus has been extensively studied in New World and Old World monkeys (Ungerleider et al., 1983; Cusick et al., 1993; Gutierrez et al., 1995, 2000; Gutierrez and Cusick, 1997; Stepniewska and Kaas, 1997; Gray et al., 1999; Stepniewska et al., 1999, 2000; Adams et al., 2000; Soares et al., 2001; Weller et al., 2002), but has been little studied to date in apes or humans. Modern studies of the pulvinar in the New World squirrel monkey and the Old World macaque monkeys suggest that these animals share a set of homologous subdivisions of the inferior pulvinar (PI) complex (Stepniewska and Kaas, 1997; Gray et al., 1999; Stepniewska et al., 1999, 2000; Adams et al., 2000; Soares et al., 2001; Weller et al., 2002), but has been little studied to date in apes or humans. Modern studies of the pulvinar in the New World squirrel monkey and the Old World macaque monkeys suggest that these animals share a set of homologous subdivisions of the inferior pulvinar (PI) complex (Stepniewska and Kaas, 1997; Gray et al., 1999). A recent study of the human PI complex (Cola et al., 1999), using histochemical techniques comparable to those used in monkeys, identified plausible homologs of most of these divisions, but suggested that one division might be lacking in humans. In the present study, we used the same histochemical techniques to examine the pulvinar of chimpanzees, and to compare the organization of the chimpanzee PI to that of humans and monkeys.

The pulvinar nucleus is the largest component of the subcortical visual system in primates, but its precise functional roles are poorly understood. Because of its extensive connections with striate and extrastriate cortical visual areas (for review, see Gutierrez et al., 2000) the pulvinar is presumed to play an important role in visual processing, including control of eye movements and directed visual attention or “visual salience” (Petersen et al., 1985, 1987; Robinson and Petersen, 1982; Bender and Youakim, 2001; Karnath et al., 2002), as well as “binding” of perceptual features (Ward et al., 2002) and semantic-lexical visual- auditory functions (Kraut et al., 2003; Crosson, 1999).

Traditionally, the pulvinar has been divided into three principal nuclei: the medial pulvinar (PM), lateral pulvinar (PL), and inferior pulvinar (PI) (Olszewski, 1952). This parcellation was based primarily on cytoarchitecture and the location of prominent fiber bundles, however, and does not correlate with retinotopic maps (Bender, 1981; Ungerleider et al., 1983; Gutierrez and Cusick, 1997). Recent studies using neurochemical approaches suggest a pattern of organization more consistent with the topography of connections with different visual areas (Cusick et al., 1993; Gutierrez and Cusick, 1997; Soares et al., 2001; Weller et al., 2002; Gray et al., 1999; Stepniewska and Kaas, 1997; Stepniewska et al., 2000). In Old World and New World monkeys, the ventral portion of the pulvinar, consisting of traditional PI and the ventral portion of traditional PL, constitutes a histochemically distinct area termed “the inferior pulvinar (PI) complex” (Cusick et al., 1993; Gutierrez et al., 1995; Gray et al., 1999). The PI complex in both squirrel and macaque monkeys consists of five neurochemically distinguishable subdivisions termed (from medial to lateral): the posterior (PIp), medial (PIm), central (PIC), lateral (PL), and lateral-shell (PLS) divisions (Gutierrez et al., 1995; Gray et al., 1999; see Stepniewska and Kaas, 1997, for a slightly different terminology). These histochemical subdivisions, which have distinctive patterns of connections with known visual areas, cross the dorsal and lateral boundaries of the traditional inferior pulvinar.

The connectional evidence suggests that the neurochemical subdivisions of PI correspond to functionally distinct zones. In monkeys, individual PI subdivisions have differential connections with visual cortex. For example, the medial subdivision, PIm, has dense connections with MT, as well as with DM, suggesting a role for PIm in the dorsal visual stream and/or in motion processing (Cusick et al., 1993; Beck and Kaas, 1998; Gray et al., 1999; Stepniewska et al., 1999, 2000; Adams et al., 2000). PIm also receives dense inputs from striate cortex, presumably originating from large motion-sensitive neurons in layer V (Gutierrez and Cusick, 1997; Rockland, 1998). In contrast to PIm, the immediately adjacent PIC connects strongly with the rostral dorsolateral area (DLr) and very little with area MT (Cusick et al., 1993; Gray et al., 1999; Weller et al., 2002). The PIC division also receives a dense projection from the superior colliculus that avoids PIm (Stepniewska et al., 1999, 2000). Compared to monkeys, the human PI complex was observed to be proportionately smaller with respect to the pulvinar as a whole, and had an overall similar staining pattern for AChE, with the exception that clear evidence for a PIp division was lacking (Cola et al., 1999).

In light of the similarities and differences between the PI complex in monkeys and humans, the present study was carried out in chimpanzees to address the following questions: 1) Can multiple neurochemical subdivisions of the PI complex also be distinguished in chimpanzees? 2) Are the histochemical staining properties of chimpanzee PI consistent with those seen in monkeys and humans? 3) Are the subdivisions of PI in chimpanzees more similar to the pattern in monkeys or humans? To answer these questions, we examined the caudal thalamus of chimpanzees with cytochrome oxidase (CO) and acetylcholinesterase (AChE) histochemistry, and immunocytochemical localization of calbindin. Based on number of divisions and fraction of the pulvinar occupied by the PI complex, our findings indicate that the inferior pulvinar of chimpanzees consists of a set of neurochemical subdivisions more comparable to those identified in monkeys than in humans.
MATERIALS AND METHODS

Chimpanzee thalami (Table 1) were obtained from the brains of eight adults with no known neurological or behavioral abnormalities housed at the New Iberia Research Center of the University of Louisiana at Lafayette. These animals died of natural causes and the postmortem-to-fixation intervals ranged from 20 minutes to an unknown interval that was less than 12 hours. Four brains were perfused postmortem, one with buffered 10% formalin and three brains with 2–4% paraformaldehyde, then blocked and postfixed for 2 days in buffered formalin plus sucrose, or buffered 2% paraformaldehyde, respectively. The remaining brains were fixed in buffered 10% formalin for 3.5–6 days, then blocked and postfixed for 1–3 days in buffered 4% paraformaldehyde. Hemithalami were dissected from the brain, immersed in 10–20% buffered sucrose solution at 4°C for 7 days, and then transferred to cryoprotectant buffer (30% sucrose, 30% ethylene glycol, 1% polyvinylpyrrolidone in 0.1 M phosphate buffer, pH 7.2–7.4; Watson et al., 1986) for 7 days at 4°C. The blocks were then placed in fresh cryoprotectant buffer and stored at −20°C. Prior to sectioning, the thalami were transferred to 40% sucrose in 0.1 M phosphate buffer (pH 7.2–7.4) at 4°C for 5–7 days, then sectioned in the coronal plane at 30 μm on a freezing microtome. Sections were then stored in cryoprotectant buffer at −20°C until processed for histochemistry.

Parallel series of sections, at 1.5 to 1.10 intervals, were processed for chemoch architecture using an antibody to the calcium-binding protein, calbindin (monoclonal, 1:4,000, Sigma, St. Louis, MO; polyclonal, 1:500, Chemicon International, Temecula, CA; Celio, 1990) and standard ABC methods (Vector kits, Burlingame, CA; Hsu et al., 1981). Acetylcholinesterase histochemistry was performed following a modification of the Geneser-Jensen and Blackstad (1971) method using acetylthiocholine iodide (Sigma) as the substrate, followed by development in 1.25% sodium sulfide, and intensification in silver nitrate. Cytochrome-oxidase histochemistry (Wong-Riley, 1979) was performed at 37°C on a rotating shaker and intensified by the addition of 0.02 M imidazole buffer (pH 7.6) to the reaction bath (Preuss et al., 1999).

Sections were viewed under brightfield and darkfield optics and low-magnification drawings were made by two independent observers using a camera lucida. Series of parallel sections were photographed using a Leaf Microlumina digitizing camera. Images were adjusted to produce sharpness and contrast comparable to gray-scale photographs. Adjacent sections were aligned as semitransparent layers using blood vessels and then corrected for differential shrinkage in Adobe Photoshop 5.5 (San Jose, CA). This procedure allowed direct comparison of the locations of candidate subdivisions as visualized with the different neurochemical methods. Brain sections processed by the same methods available from previous studies of macaque monkeys and humans (e.g., Cola et al., 1999; Gray et al., 1999) were examined for comparison.

RESULTS

Before presenting findings on the neurochemical organization of the pulvinar of chimpanzees, a brief review of our previous findings in macaques and humans is presented for comparison. In macaque monkeys, staining of the PI complex for AChE produced a series of alternating light and dark bands (Fig. 1), as described previously (Gutierrez et al., 1995; Cola et al., 1999). A similar alternating dark and light banded pattern was also visualized with cytochrome oxidase (CO) histochemistry and calbindin immunocytochemistry. The area of densest AChE stain occurred in PLm, where it formed clusters containing smaller patches of intense stain (Fig. 1). These patches appeared to fuse to form a set of curved, vertically oriented bands. In CO-stained sections (not shown), PLm also displayed patches of intense stain, although not as wide as were seen with AChE. The posterior (PLp) and central (PLc) subdivisions, located medially and laterally, respectively, were more lightly stained than PLm by both AChE and CO histochemistry. The lateral division of the PI complex, PLl, was moderately stained compared with PLm and PLc, and adjointed by a lighter shell, PLl,st. In addition, each staining method revealed divisions that extended dorsal to the brachium of the superior colliculus (bsc), and thus outside of the traditional PI nucleus.
As previously described (Cola et al., 1999), AChE (Fig. 2) and CO staining (not shown) of the human PI complex revealed a pattern of alternating dark and light bands similar to that seen in macaques. A medial region containing small patches of intense stain, comparable to PI\textsubscript{M} in macaques, was observed, bordered laterally by an area of less intense stain (PI\textsubscript{L}). Lateral to PI\textsubscript{L} was a zone of more intense staining (PI\textsubscript{L,S}), and lateral to PI\textsubscript{L,S} was a band of lighter AChE and CO stain, similar to PI\textsubscript{L,S} in macaques. Using these stains, however, Cola et al. (1999) were not able to identify in the human PI complex an area comparable to PI\textsubscript{R} of monkeys.

With regard to chimpanzees, our present observations suggest an interpretation of inferior pulvinar organization that departs significantly from previous descriptions in this species. The differences are shown schematically in Figure 3, which compares the classical parcellation of the pulvinar nuclei in chimpanzees with the chemoarchitectonic subdivisions identified in this study. Figure 3A shows the chimpanzee pulvinar nuclei as described by Walker (1938) and DeLucchi et al. (1965). Subsequent neuroanatomical studies of the chimpanzee (Armstrong, 1981; Tigges et al., 1983) have adhered to this parcellation, which was based primarily on cyto- and myeloarchitecture without the benefit of data from connectional studies. The classical PL nucleus of the pulvinar was defined by the presence of large, mediolaterally oriented fiber bundles. Rostrally, classical PL was found ventral to the brachium of the superior colliculus. Further rostrally, classical PI occupied the entire width of the pulvinar ventral to the bsc and extended \( \sim 5 \text{ mm} \) to the level of the ventroposterolateral nucleus (VPL). The dorsomedial region of the pulvinar, which lacked distinct fiber bundles, constituted the medial pulvinar nucleus PM.

In contrast to the earlier parcellation, the present chemoarchitectonic study reveals that the inferior pulvinar in the chimpanzee encompasses a region that extends more caudally than the traditional PI and includes the ventral half of traditional PL and a small, ventral portion of PM as well. Adhering to nomenclature used for similar regions in monkeys and humans, we propose the term “PI complex” for this region.

Just as in the PI complex of monkeys and humans, separate neurochemical zones were also observed in chimpanzees (Fig. 4). Calbindin (Fig. 4E–H), CO (Fig. 4I–L), and acetylcholinesterase (Fig. 4M–P) staining demarcated four histochemical zones corresponding (from medial to lateral) to the posterior (PI\textsubscript{P}; e.g., Fig. 4B,F,J,N), medial (PI\textsubscript{M} e.g., Fig. 4B,F,J,N), central (PI\textsubscript{C} e.g., Fig. 4B,F,J,N), and lateral (PI\textsubscript{L} e.g., Fig. 4C,G,K,O) divisions in monkeys. A fifth, lateralmost subdivision (PI\textsubscript{L,S}) was more readily distinguishable with calbindin and CO (e.g., Fig. 4G,K).

**PI\textsubscript{M}**

At low magnification, the medial region, PI\textsubscript{M}, had a distinct patchy appearance in each of the three neuro-
Fig. 2. A rostral-to-caudal series of sections through human pulvinar complex stained for AChE histochemistry with camera lucida drawings of PI subdivisions superimposed. Intervals between sections are 300 μm. Modified from Cola et al. (1999). Abbreviations and orientation as in Figure 1. Scale bar = 2 mm.
chemical methods used (Fig. 4D,H,L,P). At higher magnification, PIₐ appeared as a conspicuous light zone when stained for calbindin (Fig. 5A,D), with very light neuropil stain and sparse calbindin-positive neurons. By contrast, CO and AChE histochemistry revealed several intensely stained patches in this zone, resembling the pattern seen in monkeys and humans. The pattern of densest stain ranged from patches about 400 μm in diameter to elongated bands extending 3.0 mm dorsoventrally (Fig. 4D,H,K,O). This patchiness, as found in monkeys and humans, suggests a modular organization within PIₐ.

The PIₐ subdivision occupied about one-third of the total area of the classical PI nucleus and was as much as 3.5 mm wide in some individuals. The rostrocaudal extent ranged from 2.5–3.0 mm. These dimensions notwithstanding, PIₐ was not confined to classical PI. Approximately one-third to one-half of PIₐ extended dorsally across the brachium of the superior colliculus on individual sections. Moreover, PIₐ extended caudal to the bsc, beyond the caudal limit of traditional PI.

**PIₚ and PIₐ**

The PIₚ and PIₐ divisions, which are located, respectively, medial and lateral to PIₐ, displayed the heaviest calbindin immunoreactivity for both cells and neuropil in Nissl- or myelin-stained sections. The subdivisions in B are based on the neuropil and cellular staining patterns for calbindin, AChE, and cytochrome oxidase. Bsc, brachium of superior colliculus; PulI, inferior pulvinar nucleus; PulL, lateral pulvinar nucleus; PulM, medial pulvinar nucleus. Other conventions as in Figure 1.

**PIₐ**

The PIₐ subdivision in the chimpanzee was characterized by a band of intense stain demonstrated by all staining methods along the lateral border of PIₐ. PIₐ was more densely stained with CO and AChE, and more darkly stained for calbindin, than the medially adjacent area PIₚ (Fig. 4K,O). Within the lateral compartment of this zone, there was a dense population of small calbindin-stained neurons interspersed with a small number of large, darkly stained neurons (Fig. 5E). The small calbindin-stained neurons had moderately stained somas with a few lightly stained dendrites, whereas larger immunoreactive neurons had a darkly stained soma with dark radiating dendrites. While the calbindin immunoreactivity appeared homogenous in PIₐ, AChE and CO histochemistry revealed areas of slight patchiness or heterogeneity (Fig. 4J,N).

PIₐ corresponded to the entire rostral and approximately one-third of the caudal extent of classical PI, measuring ~3.0 mm in the rostrocaudal dimension. PIₐ also extended dorsal to the bsc, into traditional PL, and ended caudal to the bsc (Fig. 4L,M).

**PIₐ-S**

PIₐ was adjoined laterally by a narrow “shell” zone of lighter staining by all three methods. The intensely stained calbindin “giant” cells described in squirrel monkeys and macaques (Cusick et al., 1993; Gray et al., 1999) were also visible in this region (Figs. 6, 7). PIₐ-S extended dorsally into traditional PL, and scattered large calbindin cells were found in PL and PM. PIₐ-S was typically visible for ~1.5 mm from rostral to caudal with a width of ~1.0–2.0 mm. Just as for the other divisions, it extended above and caudal to the bsc.

**DISCUSSION**

The present investigation shows many qualitative similarities between the neurochemical organization of the PI complex of chimpanzees and that of New World monkeys, Old World monkeys, and humans. Table 2 summarizes the staining characteristics of each PI subdivision in chimpanzees for each of the neurochemical markers employed. Similar staining patterns within the PI complex across species have now been demonstrated using AChE, CO, and calbindin (Cusick et al., 1993; Gutierrez et al., 1995; Stepniewska and Kaas, 1997; Cola et al., 1999; Gray et al., 1999). As five distinct but comparable subdivisions were identified in the chimpanzee, we propose to apply the same nomenclature to this species as was used for macaques and humans (Cola et al., 1999; Gray et al., 1999). The AChE, CO, and calbindin stains reliably revealed the boundaries of four subdivisions (PIₚ, PIₐ, PIₐ, and PIₐ) in the chimpanzee PI complex. Cytochrome oxidase histochemistry and calbindin immunocytochemistry demar-
icated the boundary between \( \text{PI}_l \) and \( \text{PI}_{L-S} \) more consistently than did AChE.

Although we did not undertake quantitative morphometric comparisons, there appeared to be differences between species in the proportions of different divisions of the pulvinar. In humans, we could identify no counterpart of the \( \text{PI}_p \) division we have identified in chimpanzees and in monkeys. For reasons discussed below, we consider \( \text{PI}_p \) to likely be an extension of \( \text{PI}_c \), rather than a distinct division of \( \text{PI} \) in its own right. Under this interpretation, the lack of a \( \text{PI}_p \) in humans would represent the morphological reduction of \( \text{PI}_p-\text{PI}_c \) rather than the loss of a discrete subnucleus. Nevertheless, the existence of a clear \( \text{PI}_p \) division in chimpanzee is more similar to monkeys than to human. It is also noteworthy that the histochemical border of the PI complex, within the pulvinar as a whole, was in a proportionately more dorsal location in both monkeys and chimpanzees compared to humans. This suggests that the PI complex, interconnected with early visual areas, is larger, relative to PM and dorsal PL, in chimpanzees than in humans. The pulvinar, considered as a whole, is much larger in humans than in chimpanzees or other great apes (Armstrong, 1981), and it seems likely that the PM, which projects to many regions of higher-order association cortex, underwent a greater degree of expansion in human evolution than did the PI complex, which projects mainly to striate and extra-striate visual cortex (see Gutierrez et al., 2000, for review).

These differences in adult pulvinar structure in different primate lines may be linked to observations that the human pulvinar contains cell populations that are not found in macaques or in nonprimate mammals. Earlier histological observations indicated a telencephalic origin for some of the neurons in the human pulvinar (Rakic and Sidman, 1969; Sidman and Rakic, 1973). Building on those studies, Letinic and Rakic (2001) showed that a population of GABAergic neurons in human thalamus originates from the ganglionic eminence of the telencephalon. It may be that the evolutionary enlargement of the human thalamus, in particular the association nuclei, and especially the pulvinar, resulted from a developmental influx of neurons from the telencephalon via a migratory stream not found in other mammals (Letinic and Kostovic, 1997). Such neurons are not found in monkeys or rodents, but the status of chimpanzees in this regard has not yet been investigated. The contribution of the ganglionic eminence to the thalamus could be a human specialization, or it could be an ape-human specialization.

The distribution of calbindin-positive neurons and neuropil staining within the chimpanzee PI complex resembled that of macaque monkeys. As in other species, the \( \text{PI}_m \), the “calbindin hole” (Cusick et al., 1993) of neurochemically defined PI, contained light neuropil staining and sparse calbindin-stained neurons. This feature, together with the dense staining for CO, suggests that, like monkeys and humans, the chimpanzee \( \text{PI}_m \) has neurochemical features resembling those of primary sensory relay nuclei of the thalamus (Cusick et al., 1993) such as the ventroposterior and medial geniculate nuclei (Hashikawa et al., 1991; Rausell and Jones, 1991; Rausell et al., 1992a,b). In all of these structures, compartments or layers that are dense in CO are also poor in calbindin. This staining pattern also characterizes thalamic compartments with fast sensory conduction and secure transmission and has been termed “lemniscal” (Cusick et al., 1993).

Interestingly, the driving afferents to \( \text{PI}_m \) and from there to the motion processing area MT appear to originate from large layer V neurons in the striate cortex (Gutierrez and Cusick, 1997; Rockland, 1998; Feig and Harting, 1998).

The patchy staining of \( \text{PI}_m \) with AChE and CO found in chimpanzees agrees well with the pattern of intense and lighter stain described previously in Old World and New World monkeys (Lin and Kaas, 1979; Stepniewska and Kaas, 1997; Cusick et al., 1995; Gutierrez et al., 1995; Gutierrez and Cusick, 1997; Gray et al., 1999) and in humans (Cola et al., 1999). The consistent presence of patches in different species suggests modularity within \( \text{PI}_m \). Connectional studies in macaque and squirrel monkeys (Cusick et al., 1993; Gutierrez et al., 1995; Gutierrez and Cusick, 1997; Gray et al., 1999; Adams et al., 2000; Stepniewska et al., 1999) have shown reciprocal connections between \( \text{PI}_m \) and cortical area MT. Other connectional studies involving injections of tracers into area V1 or MT have also revealed patches of connections in \( \text{PI}_m \) (Gutierrez and Cusick, 1997; Gray et al., 1999). Thus, these separate patches of stain in \( \text{PI}_m \) may reflect discrete connectional units within this subdivision.

The zones immediately adjacent to \( \text{PI}_m \) (\( \text{PI}_p \) and \( \text{PI}_c \)) displayed dense calbindin immunoreactivity, with many darkly stained neurons and heavy staining of the neuropil. In one case immunostained for calbindin, \( \text{PI}_m \) and \( \text{PI}_p \) appeared to fuse ventrally, suggesting that they may actually be a single division. In macaque and squirrel monkeys, both \( \text{PI}_p \) and \( \text{PI}_c \) interconnect with rostral dorsolateral cortical, DLr (Cusick et al., 1993; Steele and Weller, 1993; Gray et al., 1999), and receive input from the superficial layers of the superior colliculus (Stepniewska et al., 1999, 2000), again supporting the possibility that these two zones are a single division. In light of this possibility, the apparent absence of an \( \text{PI}_p \) division in humans (Cola et al., 1999) may not represent a true difference in numbers of subdivisions, but rather a limited medial extent of a "shell" zone with respect to \( \text{PI}_m \).

The largest PI subdivision, \( \text{PI}_c \), as identified by calbindin, was densely stained and characterized by containing large darkly stained neurons within a population of smaller lightly stained neurons. Rostrally, \( \text{PI}_c \) occupied the full mediodlateral width of the inferior pulvinar, while caudally it extended to the caudal pole. \( \text{PI}_{L-S} \), the laterally adjoining subdivision, had lightly stained neuropil with calbindin, but contained numerous giant calbindin cells. \( \text{PI}_{L-S} \) is more clearly distinguishable as a zone separate from \( \text{PI}_m \) with calbindin and CO than with AChE. Connections of V1, MT, and the DL region in monkeys separately target \( \text{PI}_{L-S} \) in a retinotopically organized fashion and support its existence as a distinct division from \( \text{PI}_c \) (Cusick et al., 1993; Gutierrez and Cusick, 1997; Gray et al., 1999).

There are few existing studies of the subcortical connections of the pulvinar in chimpanzees. Walker (1939) reported that, following ablation of the occipital lobe posterior to the parieto-occipital fissure, and the posterior temporal region, retrograde degeneration occupied the traditional PL and PI. The degeneration continued rostrally in the same relative position to the anterior pole of the PI nucleus. While these results were obtained without the benefit of neurochemically defined pulvinar subdivisions or modern tracing methods, the regions of degeneration would appear to correspond to the PI complex.

Injections of horseradish peroxidase into cortical areas 17 and 18 in chimpanzee brains labeled cells in the tradi-
Fig. 4. Line drawings of chimpanzee PI subdivisions (A–D) and low power darkfield images of calbindin stained sections from the same levels (E–H). Note the staining patterns of individual subdivisions consistently rise above the bsc. I–L: PI complex shown with cytochrome oxidase and with AChE stains (M–P). Rostral-to-caudal series: The within-series spacing (e.g., A–B, B–C, C–D) is 300 μm and the between-series spacing (e.g., A–E, B–F) is 60 μm. Arrows indicate dorsal border of PI. Conventions as in Figure 2. Scale bars = 1 mm.
tional inferior and lateral pulvinar (Tigges et al., 1983). The resulting cluster of label in the inferior pulvinar was situated ventral to the bsc at the level of the posterior LGN and was confined to the area between the lateral and medial geniculate nuclei. The labeled neurons stretched rostrocaudally for ~1.5 mm and formed longitudinal
Fig. 5. Distribution of calbindin-positive neurons and neuropil staining within neurochemically
delineated PI subdivisions. Each region indicated in (A) is shown at higher magnification in the lower
panels (B–E). Conventions as in Figure 3. Scale bar = 500 μm in A; 90 μm in D (applies to B–E).
bands that extended beyond the classically defined boundaries of the inferior pulvinar. These results are in agreement with similar connectional studies in macaque monkeys (Gutierrez and Cusick, 1997). While connectional data in chimpanzees are limited (Walker, 1939; Tigges et al., 1983), the observation that connections do not conform to classical boundaries of the pulvinar is consistent with data from other species (Gray et al., 1999) and supports the present data regarding neurochemical subdivisions of the inferior pulvinar.

The present study correlated three different neurochemical methods on neighboring serial sections to define the location and internal organization of the chimpanzee inferior pulvinar complex. Separate neurochemical subdivisions within the chimpanzee PI suggest functional differences reflected by their patterns of cortical connections. In monkeys, individual subdivisions connect with several visual areas and individual visual areas connect with several subdivisions (e.g., Gutierrez and Cusick, 1997; Gray et al., 1999; Adams et al., 2000). Homologous subdivisions in chimpanzee may have similar characteristics. If the chimpanzee PI complex corresponds, as in monkeys, to the portion of pulvinar connected to retinotopically organized visual areas V1, V2, V4 (Cusick et al., 1993; Gutierrez and Cusick, 1997; Robinson and Cowie, 1997), then the PI complex might be involved in processing color, form, and motion of a visual stimulus. The dense connections of area MT in monkeys to PI (Cusick et al., 1993) suggest a role for this division in motion processing in chimpanzees as well.

Taking a broader, evolutionary perspective, the present comparative data suggest that the PI complex is a primate trait that co-evolved with motion processing areas of the superior temporal region. In all primates that have been examined with calbindin immunocytochemistry, there is a medial oval of decreased stain within the classical inferior pulvinar. This "calbindin hole" is flanked by areas of denser stain which laterally become alternating bands of medium and darker stain (Cusick et al., 1993; Gutierrez et al., 1995; Beck and Kaas, 1998; Adams et al., 2000; Soares et al., 2001; present study). Similarly, in the posterior superior temporal cortex, primates exhibit a densely myelinated motion processing area MT which is surrounded by a more lightly myelinated rim of cortex (Cusick et al., 1984; Desimone and Ungerleider, 1986). MT is connected with the "calbindin hole" while the myelin-light rim of MT is connected with the calbindin dark rim of PIL (Cusick et al., 1993; Gray et al., 1999; Weller et al., 2002). These striking patterns of thalamic histochemistry and functional connectivity are absent in common laboratory ani-
mals such as rats and the domestic cat. This further suggests a restricted evolutionary history of the inferior pulvinar complex and superior temporal lobe visual areas to the primate line.

ACKNOWLEDGMENT

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TABLE 2. Neurochemical Characteristics of Chimpanzee PI Complex

<table>
<thead>
<tr>
<th>Subdivisions</th>
<th>Cytochrome oxidase</th>
<th>AChE</th>
<th>Calbindin</th>
</tr>
</thead>
<tbody>
<tr>
<td>PI&lt;sub&gt;p&lt;/sub&gt;</td>
<td>light</td>
<td>light</td>
<td>moderate</td>
</tr>
<tr>
<td>PI&lt;sub&gt;M&lt;/sub&gt;</td>
<td>intense/patchy</td>
<td>intense/patchy</td>
<td>very light</td>
</tr>
<tr>
<td>PI&lt;sub&gt;C&lt;/sub&gt;</td>
<td>light</td>
<td>light</td>
<td>moderate</td>
</tr>
<tr>
<td>PI&lt;sub&gt;L&lt;/sub&gt;</td>
<td>dark</td>
<td>dark</td>
<td>dark</td>
</tr>
<tr>
<td>PI&lt;sub&gt;L-S&lt;/sub&gt;</td>
<td>moderate</td>
<td>moderate</td>
<td>moderate, with large darkly stained cells</td>
</tr>
</tbody>
</table>

LITERATURE CITED


Beck PD, Kaas JH. 1998. Cortical connections of the dorsomedial visual area in New World owl monkeys (Aotus trivirgatus) and squirrel monkeys (Saimiri sciureus). J Comp Neurol 400:18–34.


