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Evidence for Ape and Human Specializations in Geniculostriate Projections from VGLUT2 Immunohistochemistry

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Abstract

Vesicular glutamate transporters reuptake glutamate into synaptic vesicles at excitatory synapses. Vesicular glutamate transporter 2 (VGLUT2) is localized in the cortical terminals of neuronal somas located in the main sensory nuclei of the thalamus. Thus, immunolabeling of cortex with antibodies to VGLUT2 can reveal geniculostriate terminal distributions in species in which connectivity cannot be studied with tract-tracing techniques, permitting broader comparative studies of cortical specializations. Here, we used VGLUT2 immunohistochemistry to compare the organization of geniculostriate afferents in primary visual cortex in hominid primates (humans, chimpanzees, orangutan), Old World monkeys (rhesus macaques, vervets), and New World monkeys (squirrel monkeys). The New World and Old World monkeys had a broad, dense band of terminal-like labeling in cortical layer 4C, a narrow band of labeling in layer 4A, and additional labeling in layers 2/3 and 6, consistent with results from conventional tract-tracing studies in these species. By contrast, although the hominid primates had a prominent layer 4C band, labeling of layer 4A was sparse or absent. Label was also present in layers 2/3 and 6, although labeling of layer 6 in hominids was weaker and possibly more individually variable than in Old World and New World monkeys. These findings are consistent with previous observations from cytochrome oxidase histochemistry and a very small number of connectivity studies, suggesting that the projection from the parvocellular layers of the lateral geniculate nucleus to layer 4A were strongly reduced or eliminated in humans and apes following their evolutionary divergence from the other anthropoid primates.

Keywords

Vesicular glutamate transporter; Area 17; Primary visual cortex; Architectonics; Primate; Human; Blobs; Evolution; Chimpanzees

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Introduction

The primary visual cortex (area V1; area 17; striate cortex) receives its principal thalamic inputs from the lateral geniculate nucleus (LGN). The laminar distribution of LGN projections has been very well characterized in a variety of New World and Old World monkeys by conventional tract-tracing studies [reviewed in Casagrande and Kaas, 1994; Preuss, 2004]. In all monkey species examined, the strongest projections are to layer 4C, including sublayer 4C β , which is the target of projections from the parvocellular LGN layers, and sublayer 4C α , which receives input from the magnocellular LGN. Weaker projections from both magnocellular and parvocellular LGN layers target cortical layer 6 [Blasdel and Lund, 1983], while the koniocellular LGN layers project to the blobs in layers 2/3 [Callaway, 1998; Casagrande, 1994], and to layer 1 [Casagrande, 1994]. Also, with the sole exception of the New World owl monkey, *Aotus* [Kaas et al., 1976; Diamond et al., 1985], all the New World and Old World monkey species examined to date show a projection from the parvocellular LGN to layer 4A, which in tissue sectioned through the thickness of the cortex appears as a thin, irregular band separate from the broad band of fiber terminals that spans layer 4C. In sections cut parallel or tangential to the cortical surface, this network has a “honeycomb” appearance, consisting of bands of geniculostriate fibers and terminals surrounding fiber- and terminal-poor territories [Hendrickson, 1985].

In both New World and Old World monkeys, the cortical layers targeted by LGN projections stain for a mitochondrial enzyme, cytochrome oxidase (CO), high levels of which are thought to reflect high levels of synaptic activity in geniculostriate terminals [Carroll and Wong-Riley, 1984; Wong-Riley, 1979, 1989, 1994]. Thus, in most monkey species, there is dense CO staining in layers 6, 4C β , 4C α , 4A, and 1, as well as staining of the periodic blobs in layers 2/3 [Horton and Hubel, 1981; Horton, 1984; Hevner and Wong-Riley, 1990]. Owl monkeys are an exception, again, showing a low level of CO staining in layer 4A, consistent with the lack of geniculate projections to 4A in owl monkeys [Condo and Casagrande, 1990]. These results suggest that CO staining is a useful proxy for localizing geniculostriate projections. However, its utility in this role is somewhat compromised by the fact that CO staining is quite diffuse in the layers it stains and does not label thalamocortical terminal fibers distinctly [Wong-Riley 1989, Condo and Casagrande, 1990]. In addition, CO staining is reduced by fixation and by postmortem delay [Jones et al., 1992; Wong-Riley et al., 1997], which can complicate the interpretation of the reduction or loss of labeling in comparative studies.

Despite its limitations, the value of having a molecular marker for geniculocortical projections is highlighted by studies of the primary visual cortex of the hominid (great ape-human) group of primates. In humans, CO stains layers 6, 4C, and 1, and the blobs in layers 2/3, but (as in owl monkeys) layer 4A does not stain prominently [Horton and Hedley-Whyte, 1984; Wong-Riley et al., 1993; Preuss et al., 1999]. A very similar pattern of staining was reported in a study of apes (9 chimpanzees and 1 orangutan), which specifically noted the lack of a CO-dense layer 4A band in these animals [Preuss et al., 1999]. The low level of CO staining in layer 4A in hominids prompted the suggestion that geniculate projections to layer 4A were reduced in density, or possibly lost, in hominid evolution [Horton and Hedley-Whyte, 1984; Preuss et al., 1999; Wong-Riley et al., 1993; figure 1]. Great apes and humans also exhibit somewhat denser staining in layer 4B than do monkeys [Horton and Hedley-Whyte, 1984; Preuss et al., 1999], raising the possibility that geniculate afferents target layer 4B in these species [Preuss and Coleman, 2002].

Evaluating possible differences in geniculostriate projections between monkeys and hominids poses considerable difficulties, because opportunities to directly examine the connectivity of ape and human cortex at the appropriate level of detail are few, owing to

restrictions on invasive and terminal research in these species. There appear to be only two studies of connectivity that bear on the status of apes and humans. Tigges and Tigges [1979] used injections of tritiated amino acid to label the geniculostriate projection of a terminally ill chimpanzee, and reported labeling of layer 6 along with a single, broad band of label in layer 4, without a separate, more superficial band of labeling resembling layer 4A. Similarly, Miklossy [1992], examining degenerating axons and terminals in postmortem tissue from humans with lesions of the optic radiation, specifically noted an absence of degeneration in layer 4A.

Given the limitations on direct studies of connectivity in apes and humans, in which the invasive and terminal experimental procedures required for conventional tract-tracing are prohibited, and the fact that CO does not label geniculostriate fibers and terminals distinctly, it is useful to consider additional methods for labeling geniculostriate projections. One candidate is immunolabeling of parvalbumin, which is localized in geniculate projection neurons [Jones and Hendry, 1989] and shows differences in its laminar distribution in humans and macaques similar to those observed with CO, as can be seen by comparing figures 1 and 2 of Blümcke et al. [1990]. However, parvalbumin immunohistochemistry labels many processes and cell bodies intrinsic to V1, in addition to thalamocortical terminals [Blümcke et al., 1990], complicating the interpretation of species differences in geniculostriate projections.

A more promising approach to revealing geniculostriate terminals is immunohistochemistry for vesicular glutamate transporter 2 (VGLUT2). VGLUTs are transporters that reuptake glutamate into synaptic vesicles. The distributions of two isoforms, VGLUT1 and VGLUT2, have recently been examined in the thalamus and cortex of mammalian species. Both are reported to be expressed in thalamic nuclei [Barroso-Chinea et al., 2007] and thalamocortical projection neurons [Graziano et al., 2008], but other reports indicate that VGLUT1 may be restricted to corticocortical and corticothalamic synapses [Gil et al., 1999; Kaneko and Fujiyama, 2002; Kaneko et al., 2002, Nahmani and Erisir, 2005]. By contrast, there is clear evidence that VGLUT2 is strongly expressed in the primary sensory nuclei of the thalamus, including the LGN, ventral posterior nucleus (VPN), and medial geniculate nucleus (MGN) [ferret LGN: Kawasaki et al., 2004; rat MGN: Barroso-Chinea et al., 2007; mouse VPN: Graziano et al., 2008]. Cortical labeling in these studies revealed coarse fibers with prominent *en passant* terminals located mainly in the middle cortical layers, consistent with the location and morphology of primary sensory thalamocortical fibers [Nahmani and Erisir, 2005; Graziano et al., 2008; Hackett and de la Mothe, 2009]. In a direct validation of VGLUT2 as a marker for geniculostriate projections, Nahmani and Erisir [2005] injected anterograde tracer in the LGN of ferrets and showed that labeled fibers in striate cortex were immunostained for VGLUT2. Further evidence for geniculostriate localization of VGLUT2 comes from studies of mRNA expression, showing VGLUT2 expression in thalamic nuclei [galago LGN: Balaram et al., 2011; owl monkey MGN and LGN: Hackett et al., 2011], along with expression in the corresponding primary sensory cortical areas [mouse: Graziano et al., 2008; macaque: Hackett and de la Mothe, 2009].

Given the strong evidence that immunohistochemistry for VGLUT2 selectively labels geniculostriate fibers, we compared the laminar distribution of VGLUT2 immunoreactivity of primary visual cortex in humans and a variety of nonhuman primate species. The hominid primates (humans and great apes) in our sample showed much less terminal-like labeling in layer 4A, consistent with previous work suggesting that the LGN projections to this layer were reduced or lost early in hominid evolution. This reduction or loss of projections to 4A appears to constitute a hominid specialization (apomorphy).

Materials and Methods

Subjects and Tissue

We examined occipital lobe tissue from anthropoid primates of six species, including humans (*Homo sapiens*; n=5), chimpanzees (*Pan troglodytes*; n=4), 1 orangutan (*Pongo pygmaeus*; n=1), rhesus macaques (*Macaca mulatta*; n=3), vervet monkeys (*Cercopithecus aethiops*; n=3), and squirrel monkeys (*Saimiri sciureus*; n=3) (table 1). Four human specimens were obtained from the brain bank of the Northwestern University Alzheimer's Disease Center (NWADC), and 1 was from Tulane University. Age at death ranged from 56—78 years (median age = 74 years). All 5 were rated by the supplying institution as normal control brains. Postmortem delays ranged from 3–23 h (median = 6 h); brains were fixed by immersion in 10% formalin or 4% paraformaldehyde. Chimpanzee and orangutan tissue came from New Iberia Research Center at the University of Louisiana at Lafayette. Chimpanzee ages ranged from 19 to approximately 30 years; all died from natural causes or were euthanized for humane reasons. Post-mortem delays for chimpanzees were from 0–5.5 h (median 5 h); 3 brains were immersion fixed in paraformaldehyde solution, one was perfusion-fixed (Table 1). The orangutan was a 33-year-old male; the brain was immersion fixed following a postmortem delay of 2 h. Rhesus macaque brains were obtained from Yerkes National Primate Research Center; with the exception of one rhesus macaque from Vanderbilt University; ages ranged from 4–12 years (median = 6 years). The macaque brains were perfusion-fixed following post-mortem delays from 0 – <1 h (median = 0 h). Vervet monkey brains were obtained from the New Iberia Research Center; vervets ranged in age from 7 to 15 years (median = 13) and were perfused with paraformaldehyde solution. Tissue from squirrel monkeys came from the New Iberia Research Center. Age at death ranged from 18 months to 12 years (median age = 2 years). Three individuals were perfusion-fixed with phosphate-buffered (PB) paraformaldehyde, and one was immersion fixed immediately after death in PB paraformaldehyde. All procedures involving nonhuman primates were carried out according to institutional animal welfare guidelines.

Following fixation, brain blocks were cryoprotected by successive immersion in sucrose solutions of increasing concentration (10%, 20%, 30%) to prevent freezing artifact, and then stored in ethylene glycol-based cryopreservative solution [Watson et al., 1986] at –20°C. Prior to sectioning, blocks were immersed in buffered 40% sucrose at 4°C for several days to remove cryopreservative. Tissue was cut on a freezing microtome in either 40 µm (humans) or 50 µm (apes and monkeys) sections and stored in cryopreservative at –20°C before histological processing. Human brains were cut in coronal, horizontal or oblique longitudinal sections, chimpanzee brains in horizontal or coronal sections, and other species in coronal sections (table 1).

Immunohistochemistry

Purified mouse monoclonal antibody (clone 8G9.2; MAB5504), raised against recombinant rat VGLUT2, was obtained from Millipore (Temecula, CA). The epitope is unknown. This antibody has been previously used in studies of mammalian cortex [Hrabovszky et al., 2006a, 2006b; Sadakata et al., 2007; Hackett and de la Mothe, 2009; Wong and Kaas, 2009].

Tissue sections containing area V1 were first immersed with 3% H₂O₂ to inactivate endogenous peroxidase, then blocked with horse serum. Sections were then incubated in primary antibody for 2 hours at room temperature followed by biotinylated anti-mouse IgG secondary antibody for 1 hour and then a solution of biotinylated peroxide + avidin (VECTOR ABC reagent) for 1 hour as part of the Vector Elite Kit (Vector Laboratories, Inc., Burlingame, CA). Sections were stained with diaminobenzidine (DAB) solution using the Vector DAB peroxidase substrate kit (Vector Laboratories, Inc., Burlingame, CA).

Staining intensity varied across species and individuals, so series of dilution trials were carried out to yield comparable levels of staining across cases. Final primary antibody dilutions, which ranged from 1:3,000 to 1:100,000, were chosen based on the optimal ratio of specific to non-specific labeling. Samples were immunoreacted with antibody concentrations in the higher range and additional trials were run until non-specific DAB accumulation was low enough for the clear disambiguation of non-specific and specific labeling. Optimal concentrations varied between species and individuals (see Table 1). Control sections were prepared and incubated in all experiments in an identical fashion to the experimental sections, except for the omission of the primary antibody during incubation. No specific staining was observed in control sections. Sections were mounted on slides and dried using graded xylene solutions. A Nissl counterstain with thionin was applied to selected sections in order to verify the laminar distribution of labeling. Slides were coverslipped with mounting media (Cytoseal XYL; Richard-Allan Scientific, Kalamazoo, MI) to prepare for digital scanning.

Image Processing and Laminar Analysis

Digital images were acquired using a Scanscope digital slide scanner (Aperio, Vista, CA). Adobe Photoshop software (Adobe Systems, Mountain View, CA) was used to adjust contrast levels and, in the Nissl-counterstained sections, to separate the blue Nissl staining from the red-brown anti-VGLUT2/DAB staining by splitting color channels, as described by Preuss et al. [1999]. DAB chromogen was visualized using the yellow channel in CMYK color space or the blue channel in RGB space; thionin was visualized using the red channel in RGB color space. Area V1 cortical layers were identified and numbered according to the system of Brodmann [1909], as modified by Lund [1973]. Some authorities [e.g., Casagrande et al., 2007] prefer the system of Hässler and Wagner [1965], whose layers 3B β , 3C, 4 α , and 4 β correspond to layers 4A, 4B, 4C α , and 4C β , respectively, of Lund [Billings-Gagliardi et al., 1974].

Results

Immunostaining with anti-VGLUT2 yielded strong staining of area V1 in all species examined. Dense label was present throughout area V1, ending abruptly at the border of areas V1 and V2 (figure 2). In all species, the densest label was present in layers 4 and 6, although some label was present in all layers (figures 2—5). Fixation did not appear to affect VGLUT2 labeling, as evidenced by similar staining across all chimpanzee cases, which included both immersion- and perfusion-fixed tissue (figure 3). Although post-mortem delay (PMD) varied markedly within our hominid sample, immunolabeling was also not noticeably affected: compare chimpanzee N98-47 (1h PMD) in figures 3b and 5e with chimpanzee N99-11 (5h PMD) in figure 4c. Two types of staining were distinguishable: light-to-moderate diffuse staining of tissue, the appearance of which is consistent with non-specific DAB accumulation, and darker staining of discrete, terminal-like processes (figure 6). We focus here on the terminal-like staining and its laminar distribution across species.

All species examined exhibited dense terminal-like labeling in layer 4C, including both its deep (4C β) and superficial (4C α) parts, although labeling was densest in layer 4C β (figures 2—5, 6a). In 4C β of most species, labeling was concentrated in vertical stacks or arrays, although this was less apparent in squirrel monkeys than in other species (figure 5). In 4C α , by contrast, many of the terminal-like processes had oblique or horizontal orientations, as well as vertical.

Labeling of layer 4A varied markedly across species. In squirrel monkeys, macaques, and vervets, a thin band of labeling was observed at low magnification (figures 2a; 4a, 4b; 5a, 5b, 5c). At higher magnification, this band could be seen to be composed of terminal-like

staining (insets in figure 5a—c, and figure 6b). The terminal-like fibers were distributed in all orientations, and in some sections, they appeared to be concentrated in clusters distributed parallel to the pial surface. In some sections, sparse, small, labeled puncta could be observed in portions of 4A that appeared to represent individual *en passant* boutons (figure 6b). In chimpanzees and humans, by contrast, very little labeling was observed in layer 4A, with only a few terminal-like processes observed at high magnification (figure 5e—f, insets). Further, most of these processes were vertically oriented, suggesting that these consisted at least in part of fibers passing to the superficial layers. The single orangutan we studied (figure 5d) had somewhat more terminal-like labeling of layer 4A than the chimpanzees and humans, although labeling was much sparser in the orangutan than in any of the monkeys.

Deep to layer 4, label was observed in layer 6, particularly in its upper part (layer 6A), but the strength of labeling varied between species and individuals. Overall, labeling of layer 6 was denser and more consistent in the monkeys than in the hominids (compare the squirrel monkey and human in figures 2a and 2b, and the monkeys in figures 4a and 4b to the chimpanzee and human in figures 4c and 4d). The orangutan also had weak labeling of layer 6 (figure 6c). There was also substantial individual variation in labeling of layer 6 in both humans and chimpanzees, as illustrated by the moderate labeling of the human shown in figure 2b compared to the near absence of labeling in the human shown in figure 4d. Two of our 4 chimpanzee specimens showed a distinct band of labeling in layer 6 (e.g., N98-47, figure 3b), one showed faint, patchy labeling (N99-11, figure 4c), and one displayed little or no layer 6 labeling. Of the five humans, only one showed conspicuous labeling of layer 6 (N01-15, figure 2b); in the other four cases, layer 6 labeling was either very sparse or absent (e.g., N99-9, figure 4d). Terminal-like labeling in layer 6 was not uniformly distributed, but appeared to be clustered in the plane parallel to the pia.

In most cases, sparse labeling of terminal-like processes was observed in layer 3, with a preponderance of vertical and oblique orientations. In a few cases (squirrel monkeys N98-7 and N98-10, vervet monkeys N96-17 and N96-19), and chimpanzees, N96-30, N98-40, and N96-29 (the latter pictured in figure 7a and 7b) these processes appeared to form broad (~100–200 μ m wide), vertically extended clusters, which resemble blobs in both location and size. In some cases, we also observed terminal-like processes in layers 2 and 1, that in all instances consisted of sparse and sporadic labeling resembling *en passant* terminals (vervet monkeys N96-20, N96-19, and N96-17; orangutan N96-40; chimpanzees N99-11 and N96-30, and humans N98-32 and N99-13; N98-32 pictured in figure 7c).

Discussion

VGLUT2 immunohistochemistry as a tool for labeling geniculostriate afferents in postmortem material

The distribution of VGLUT2 labeling of primary visual cortex documented in our study closely matches published accounts of the distribution of geniculostriate projects in several dimensions of organization. At high magnification, the elements that stain strongly for VGLUT2 appear to be fibers studded with thickenings resembling *en passant* synaptic boutons, an appearance consistent with the geniculostriate terminals labeled by tract-tracing studies in several species [rhesus macaque: Blasdel and Lund, 1983; pigtail macaque: Freund et al., 1989; galago: Florence and Casagrande, 1987; cat: Ferster and Levay, 1978; Mason and Robson, 1979; Humphrey et al., 1985; ferret: Nahmani and Erisir, 2005]. The study by Nahmani and Erisir [2005] is especially notable, because they found that the geniculostriate fibers and terminals labeled with anterograde tracer injections of the LGN were also strongly VGLUT2 immunopositive. Second, the dense VGLUT2 immunostaining found in area V1 ended abruptly at the V1/V2 border, and in primates, V1 is known to

project very strongly to V1 but only minimally to V2 [reviewed in Preuss, 2007]. Finally, as discussed in more detail below, the laminar and modular organization of VGLUT2 labeling within area V1 closely matches published descriptions of the tangential and radial distribution of geniculostriate projections. It is therefore highly likely that the terminal-like labeling we observed in striate cortex with VGLUT2 immunostaining included predominantly geniculostriate fibers, although it remains possible that it does not represent exclusively geniculostriate fibers.

These results demonstrate the value of VGLUT2 immunohistochemistry as a proxy or marker for geniculate terminals in great apes and humans, species in which invasive tract-tracing studies cannot ordinarily be carried out. Furthermore, this technique would seem to provide less ambiguous evidence for changes in geniculostriate projections than is possible with cytochrome oxidase (CO) histochemistry or with parvalbumin (PV) immunohistochemistry, both of which produce relatively diffuse labeling in contrast to the well-defined, terminal-like labeling observed with anti-VGLUT2 in area V1. The distribution of VGLUT2 immunoreactivity across the layers of area V1 corresponds closely to the distribution of CO and PV the cortical layers that label with anti-VGLUT2, however, and thus results with anti-VGLUT2 reinforce interpretations of visual cortex organization and evolution obtained with CO and PV.

Laminar and radial distribution of VGLUT2 immunoreactivity: similarities and differences across species

The laminar distribution of terminal-like VGLUT2-immunopositive labeling of the primary visual area shared several characteristics across different groups of primates, and matched well the main targets of geniculostriate projections identified in tract-tracing studies [Blasdel and Lund, 1983; Casagrande, 1994; Callaway, 1998; reviewed in Casagrande and Kaas, 1994; Preuss, 2004, 2007]. In the New World and Old World monkeys, as well as in the hominids, the densest VGLUT2 labeling was present in layer 4C, spanning its lower (4C β) and upper (4C α) strata, which are the targets of projections from the parvocellular and magnocellular LGN layers, respectively. Terminal-like labeling was also present in layer 6 in all groups, although the density of staining was variable: the monkeys consistently displayed dense labeling in this layer, while the hominids were more lightly labeled overall and showed conspicuous inter-individual variability. Labeling of layer 4A showed even more striking differences across species: New World and Old World monkeys had terminal-like labeling in a thin, irregular stratum, but labeling of this layer ranged from sparse to absent in apes or humans. Lastly, small numbers of terminal-like processes were observed in layers 1, 2, and 3 in most cases. At high magnification, labeling often took the form of a series of thickenings strung along a fiber, suggesting *en passant* terminals (figure 7c). In addition, large clusters of label in layer 3 were observed in some cases, resembling the blobs observable in tissue stained for cytochrome oxidase [Horton and Hubel, 1981; Horton, 1984; Hevner and Wong-Riley 1990]. It is known that the LGN projects directly to blobs in monkeys [Livingstone and Hubel, 1982; Fitzpatrick et al., 1983; Weber et al., 1983; Lachica and Casagrande, 1992; Ding and Casagrande, 1997]. We note, however, that our material was not sectioned in the manner most useful for demonstrating blobs, that is, in planes of section tangential or parallel to the surface of the cortex.

With regard to layer 4A, the present comparative results obtained with VGLUT2 immunohistochemistry mirror results obtained previously with cytochrome oxidase (CO) histochemistry [Preuss et al., 1999]: as with CO, staining for VGLUT2 revealed no obvious dense band of labeling in this layer, in contrast to the Old World and New World monkeys. Thus, both the VGLUT2 and CO data support the hypothesis that parvocellular LGN projections to layer 4A were markedly reduced or lost in hominid evolution [Preuss et al., 1999; see also Horton and Hedley-White, 1984, and Wong-Riley et al., 1993]. Preuss et al.

[1999] also indicated that CO staining of layer 4B might be stronger in hominids than in other anthropoid primates, suggesting that geniculate projections might extend into layer 4B in hominids, but the current results do not support this, as the hominids had no more labeling of layer 4B with anti-VGLUT2 than did the other primates examined, with the possible exception of the single orangutan in our study.

The loss of the geniculate afferents to layer 4A in hominids could be taken as evidence that layer 4A itself was lost in hominid evolution, as suggested by Horton and Hedley-Whyte [1984] after noting the absence of a band of CO staining corresponding to layer 4A in humans. On current evidence, however, we prefer the hypothesis that layer 4A was modified, rather than lost, in human evolution. Layer 4A was originally identified in Brodmann [1909] in humans and other primates based on shared cytoarchitectonic characteristics. Moreover, loss of LGN afferents is just one of several changes that occurred in this stratum in hominid evolution, changes that involved the addition of features. Specifically, in humans and apes, but not in other primates, layer 4A contains a dense population of small neurons that express the calcium-binding protein, calbindin [Preuss and Coleman, 2002]. In addition, Preuss et al. [1999] and Preuss and Coleman [2002] found that layer 4A in humans, but not other hominids or anthropoids, is enriched in molecules that have been associated with the magnocellular visual pathway, specifically aggrecan, the extracellular matrix proteoglycan labeled by the Cat-301 antibody [Fryer et al., 1992], non-phosphorylated neurofilament (which is labeled by antibody SMI-32), and microtubule-association protein 2 (MAP2). These human specializations could well indicate an enhancement or reorganization of magnocellular processing in human striate cortex, although the absence of VGLUT2 or CO staining in layer 4A makes it unlikely any such modification involved the evolution of a direct projection from the magnocellular LGN to that layer. Because V1 is the main source of visual information for higher-order areas, changes in V1 organization could affect higher levels of visual-system function [Preuss, 2004]. Changes to area V1 in hominid evolution could be reflected in known increases in motion sensitivity in human areas V3 and V3A [Tootell et al., 1997] and in human intraparietal areas [Vanduffel et al., 2002], compared to their macaque homologues, and in the selective responsiveness of human anterior inferior parietal cortex to the sight of tool actions, something not observed in macaques [Peeters et al., 2009].

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Figure 1.

Phylogenetic tree showing the evolutionary relationships among the species examined in this study, sample sizes, and the distribution of character-state changes in LGN projections inferred from previous research. The present study provides additional evidence to support state change 2, the reduction or loss of direct projections from the parvocellular LGN to layer 4A after the divergence of the Hominidae and Cercopithecoidea.

Figure 2.

The marked reduction of VGLUT2 immunolabeling at the transition between areas V1 and V2 is illustrated in (a) a squirrel monkey (*Saimiri*) and (b) a human (*Homo*). The arrowheads in (a) mark the location of the layer 4A labeling band. Scale bars = 250 μ m.

Figure 3. Anti-VGLUT2 DAB labeling of a perfusion-fixed chimpanzee (a) and an immersion-fixed chimpanzee (b). Scale bars = 100 μm.

Figure 4.

The laminar distribution of VGLUT2 immunolabeling in area V1 depicted in: (a) squirrel monkey (*Saimiri*), (b) rhesus macaque (*Macaca*), (c) chimpanzee (*Pan*), and (d) human (*Homo*). Each figure pair represents a VGLUT2 immunostained section that was counterstained for Nissl with thionin. After scanning, the color channels were separated as described in the text to produce separate images of the blue Nissl staining (the left figure in each pair) and the red-brown VGLUT2 immunolabeling (the right figure in each pair). Note the lack of VGLUT2 labeling in layer 4A of the hominids (c, d) compared to the monkeys (a, b), as well as the weak labeling of layer 6 in the hominids compared to the monkeys. Scale bars = 250 μ m.

Figure 5.

Detail of VGLUT2 labeling in layer 4 of (a) squirrel monkey (*Saimiri sciureus*), (b) vervet monkey (*Cercopithecus aethiops*), (c) rhesus macaque (*Macaca mulatta*), (d) orangutan (*Pongo pygmaeus*), (e) chimpanzee (*Pan troglodytes*), and (f) human (*Homo sapiens*). Higher magnification of 4A labeling shown in insets. Main scale bars = 100 μ m, inset scale bars = 25 μ m.

Figure 6. Detail of cortical layers with terminal-like labeling in (a) layer 4C of rhesus macaque (*Macaca*), (b) layer 4A of vervet monkey (*Cercopithecus*), and (c) layer 6 of orangutan (*Pongo*). Scale bars = 100 μ m.

Figure 7.

Detail of VGLUT2 immunolabeling in the superficial layers of area V1. (a) Arrows denote centers of blob-like labeling in layer 3 in a chimpanzee. (b) Higher magnification of the left blob-like territory shown in (a). (c) A labeled, vertically oriented fiber in layer 3 of a human showing apparent en passant terminal boutons. Scale bars = 250 μm (a); 125 μm (b); 10 μm (c).

Table 1

Summary of cases examined.

Species	Case	Age	Sex	Fix	Plane; Thickness	PMD	Cause of Death	VGLUT2 conc.
<i>Homo sapiens</i>	N98-32	78	M	Imm	Coro; 40 µm	23h	Lung cancer	1:3,000
	N01-15	U	U	Imm	Long; 40 µm	U	U	1:25,000
	N99-6	70	F	Imm	Coro; 40 µm	3h	Cancer	1:10,000
	N99-13	56	F	Imm	Horiz; 40 µm	6h	Pyelonephritis	1:30,000
	N99-9	78	F	Imm	Coro; 40 µm	6h	Aortic aneurysm	1:80,000
<i>Pan troglodytes</i>	N96-29	30	F	Imm	Coro; 50 µm	5.5h	U	1:10,000
	N96-30	20	M	Perf	Horiz; 50 µm	0	Euthanasia	1:20,000
	N98-47	25	F	Imm	Horiz; 50 µm	1h	Peritonitis	1:20,000
	N99-11	19	M	Imm	Horiz; 50 µm	5h	Pneumonia	1:5,000, 1:7,000
<i>Pongo pygmaeus</i>	N98-40	~33	M	Imm	Coro; 50 µm	2h	Cardiac arrest	1:5,000
<i>Macaca mulatta</i>	N02-7	12y5m	F	Perf	Coro; 50 µm	0	Sacrifice	1:25,000
	N98-23	4	F	Perf	Coro; 50 µm	<1h	Sacrifice	1:20,000
	Y07-176	6	F	Perf	Coro; 50 µm	0	Euthanasia	1:10,000
<i>Cercopithecus aethiops</i>	N96-17	15	F	Perf	U	0	Sacrifice	1:100,000
	N96-19	7	F	Perf	U	0	Sacrifice	1:5,000
	N96-20	13	F	Perf	U	0	Sacrifice	1:3,000
<i>Saimiri sciureus</i>	N98-7	1y6m	M	Perf	Coro; 50 µm	0	Sacrifice	1:20,000
	N98-8	12	M	Perf	Coro; 50 µm	0	Sacrifice	1:20,000
	N98-10	2	M	Perf	Coro; 50 µm	0	Sacrifice	1:20,000

Fix: mode of fixation; Perf = perfusion-fixed; Imm = immersion-fixed; Coro: coronal; Long: longitudinal; Horiz: horizontal; PMD = post-mortem delay prior to fixation; VGLUT2 conc. = optimal anti-VGLUT2 antibody concentration; U = unknown.