Research Article

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Areal specialization of pyramidal cell structure in the visual cortex of the tree shrew: a new twist revealed in the evolution of cortical circuitry

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Abstract Cortical pyramidal cells, while having a characteristic morphology, show marked phenotypic variation in primates. Differences have been reported in their size, branching structure and spine density between cortical areas. In particular, there is a systematic increase in the complexity of the structure of pyramidal cells with anterior progression through occipitotemporal cortical visual areas. These differences reflect areaspecific specializations in cortical circuitry, which are believed to be important for visual processing. However, it remains unknown as to whether these regional specializations in pyramidal cell structure are restricted to primates. Here we investigated pyramidal cell structure in the visual cortex of the tree shrew, including the primary (V1), second (V2) and temporal dorsal (TD) areas. As in primates, there was a trend for more complex branching structure with anterior progression through visual areas in the tree shrew. However, contrary to the trend reported in primates, cells in the tree shrew tended to become smaller with anterior progression through V1, V2 and TD. In addition, pyramidal cells in V1 of the tree shrew are more than twice as spiny as those in primates. These data suggest that variables that shape the structure of adult cortical pyramidal cells differ among species.

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Introduction
Systematic differences have been demonstrated in pyramidal cell structure in primates, with cortical areas, which are believed to be important for normal visual processing (see Elston 2002 for a review). For example, the dendritic arbours of pyramidal cells become progressively larger, more branched and more spiny in the primary visual area (V1), the second visual area (V2), and cortical visual areas of the parietal and temporal lobes (Land et al. 1993; Elston and Rosa 1997, 1998, 2000; Elston et al. 1999a, b; Elston 2003). These specializations in structure are thought to subserve specific aspects of visual processing at both the cellular and systems levels. For example, by virtue of their highly branched, spiny dendritic arbours, pyramidal cells in inferotemporal cortex (IT) are likely to process larger numbers of excitatory inputs than cells in V1. In addition, processing of these inputs may be compartmentalized to a greater degree in the more branched dendritic arbours of cells such as those in IT as compared to those in V1.

Recently we showed that the visual cortex of the prosimianGalago is also characterized by regional specialization in pyramidal cell structure: dendritic arbours become progressively larger and more spiny from V1 to V2, the dorsolateral area (DL) and IT (Elston et al. 2005). Thus we concluded that areal specialization of pyramidal cell structure was present in a common simian/prosimian ancestor. However, it remains to be determined if this may be a characteristic of visual cortex in all mammals. In the present study we examined pyramidal cell morphology in visual areas of the tree shrew (Tupaia glis), a close relative of the primates (Cronin and Sarich 1980; Luckett 1998). We found that regional variation in pyramidal cell structure in the tree shrew differs from that in primates.
Methods

Two adult tree shrews (Tupaia glis) (one male, 38 months old, and one female, 28 months old) were anesthetized by i.n. injection of Sodium Pentobarbital. The animals were then perfused transcardially with 0.95% saline in 0.1 M phosphate buffer (PB: pH 7.2) followed by 4% paraformaldehyde in PB. The brains were removed, the right hemispheres were flat-mounted and left in the 4% paraformaldehyde in PB for 12 h at 4°C. Sections (250 μm) were cut with the aid of a Vibrotome and were then prelabelled with 10–7 mol/L 6-diamidino-2-phenylindole (D9542, Sigma, USA) in PB (approximately 5 min at room temperature). Cells were injected with 5% Lucifer Yellow (L-0259, Sigma) in 0.1 M Tris buffer (pH 7.4) by continuous negative current under visual control (UV excitation). The sections were then processed with an antibody to Lucifer Yellow (LY) at a concentration of 1:400,000 in stock solution (2% bovine serum albumin (Sigma A3425), 1% Triton X-100 (BDH 3632), 5% sucrose in 0.1 mol/L phosphate buffer) for 5 days at room temperature. The sections were then washed in PB and processed in a solution containing a species-specific biotinylated secondary antibody (Amersham RPN 1004; 1:200 in stock solution) for 2 h at room temperature, washed in PB, and processed for a further 2 h in 1:200 biotin-horseradish peroxidase complex (Amersham RPN1051) in 0.1 mol/L phosphate buffer, 3,3’-diaminobenzidine (DAB) (Sigma D 8001) was used as the chromogen (Fig. 1). Adjacent serial sections were processed for cytochrome oxidase to reveal aspects of cortical organization (Wong-Riley 1979). By aligning blood vessels in serial sections we were able to determine the location of the V1 border with a high degree of accuracy (e.g., Fig. 2 of Elston and Rosa 1998).

Cells were drawn in two dimensions, the dendritic field size determined (the area contained within a polygon joining the outermost distal tips of the basal dendrites: Elston and Rosa 1997), the branching pattern assessed by Sholl analysis (Sholl 1953) and spine density determined by counting the number of spines as a function of distance from the cell body to the distal tips of the dendrites (e.g., Eayrs and Goodhead 1959). Somal areas, like basal dendritic areas, were determined with the aid of NIH-Image software (NIH, Bethesda, US). Cells were then allocated to cortical areas by multiple criteria. The primary visual area (V1) was easily distinguishable in the section in which we injected cells, and in adjacent serial sections that were processed for cytochrome oxidase (Fig. 2), by virtue of its relatively dark diaminobenzidine reaction product. Several extrastriate visual areas have been identified by multiple criteria, including cytoarchitecture, myeloarchitecture, electrophysiological mapping, patterns of connectivity and Cat-301 labelling (Kaas et al. 1972; Sesma et al. 1984; Juin et al. 1994; Lyon et al. 1995). The second visual area (V2) is a continuous belt approximately 1.2 mm wide immediately adjacent to the anterior V1 border. Anterior to V2 lie the temporal dorsal (TD), the temporal anterior (TA) and temporal posterior (TP) areas, although the exact location of the borders of these areas is less certain. Cortex anterior to these areas has been tentatively labelled as temporal inferior area (TI) and temporal posterior inferior (TPI) areas (Lyon et al. 1998). Because we injected cells in rows from the occipital pole to the temporal pole, cells anterior to V2 were likely to be located mostly in area TD (Fig. 3). The total number of spines found in the basal dendritic arbour of the “average” pyramidal cell in each cortical area was calculated by multiplying the average number of spines of a given portion of dendrite by the average number of branches for the corresponding region, over the entire dendritic arbour (Elston 2001). All statistical analysis was performed with SPSS (SPSS Inc., Chicago, IL, USA).

Fig. 1 Photomicrographs of pyramidal cells that were injected at the base of layer III in visual cortex of the tree shrew. Neurones were injected with Lucifer Yellow and processed by standard immunohistochemical procedures for a light-stable DAB reaction product. Scale bar = 80 μm.

Results

We injected 206 neurones in layer III of V1, V2 and TD, 116 of which were included for analyses as they had an unambiguous apical dendrite, were well filled, and had
their entire basal arbours contained within the slice (50 in V1, 32 in V2 and 34 in TD).

Basal-dendritic arbour size

From Fig. 1 it can be seen that the frequency distributions of the size of the basal dendritic arbours were similar for cells in V1, V2 and TD. There was a tendency for a slight decrease in the size of the arbours with progression through these areas, but analyses of variance revealed no significant difference in either TS1 (F(2, 5) = 0.763, p = 0.479) or TS2 (F(2, 5) = 1.244, p = 0.293).

Complexity of the basal dendritic arbours

In both cases there was a trend for an increase in the peak dendritic complexity of cells with progression through areas V1, V2 and TD, the extent of which was less in TS1 (V1: 22.5 ± 6.09; V2: 23.0 ± 4.06; TD: 24.12 ± 4.14) than in TS2 (V1: 23.25 ± 4.34; V2: 27.26 ± 4.25; TD: 31.30 ± 4.13) (Fig. 4). Repeated measures ANOVAs revealed the branching patterns of cells to be significantly different between cortical areas in TS2 (intercept: F(2, 5) = 2.33 × 104, p < 0.001; cortical area: F(3, 5) = 11.8, p = 0.001), but not TS1 (intercept: F(2, 5) = 32.12, p < 0.001; cortical area: F(2, 5) = 0.02, p = 0.980). Post-hoc Scheffe tests revealed all comparisons to be significant in TS2 except for V1/V2.

Spine densities of the basal dendrites

Over 6,500 dendritic spines were drawn and tallied in areas V1, V2 and TD in TS2, in which we injected most neurons. The peak average spine density of cells in V1 (11.06 ± 2.76) was greater than that in V2 (10.65 ± 1.87) but less than that in TD (14.73 ± 3.23) (Fig. 4). A repeated measures ANOVA revealed a significant difference in the distribution of spines along the dendrites of pyramidal cells in the different cortical areas (intercept: F(2, 5) = 1.82 × 103, p < 0.001; cortical area: F(3, 5) = 37.6, p < 0.001). Post-hoc Scheffe tests revealed all between-area comparisons to be significantly different. By combining data from the Sholl analyses and spine density counts, we calculated the total number of dendritic spines in the basal dendritic arbour of the “average” layer III pyramidal neuron. Cells in V1 (1,507) were, on average,
more spiny than those in V2 (1.484), but less spiny than those in TD (2.531).

Somata areas

In both TS1 and TS2 there was a trend for a decrease in average soma size through V1, V2 and TD (Fig. 4). However, analyses of variance revealed the differences not to be significant (TS1: $F_{2,16} = 0.58$, $p < 0.57$; TS2: $F_{2,16} = 3.62$, $p < 0.052$).

Discussion

Tree shrews have been grouped variously as primates (Simpson 1945; Le Gros Clark 1959; Mahe 1976) or close relatives of primates (Campbell 1980; Conin and Sarich 1980; Luckett 1980; Martin 1990; MacPhee 1993; Simmons 1993; Springer and de Jong 2001). They are now grouped as the sole members of the order Scandentia, which, together with the orders Chiroptera (bats), Dermoptera (gliding lemurs) and primates, are grouped into the taxon Archonta (Nowak 1999). Tree shrews are highly visual mammals and their visual cortex shares many affinities with that of primates (see Lund et al. 1983 for a review). For example, the topographic organization of visual cortex in tree shrews is similar to that in monkeys (Kaas et al. 1972; Humphrey et al. 1980a, b), as are its callosal connections (Cusick et al. 1985), intrinsic axon patches (Rockland and Lund 1982; Rockland et al. 1982; Sesma et al. 1984; Bosking et al. 1997; Lyon et al. 1998) and pattern of labelling for CAT-301 (Jain et al. 1994). However, many differences between the visual systems of tree shrews and primates have been documented. For example, there is a cleft in the granular layer of V1 in tree shrew not observed in primates; its V1 lacks the characteristic blobs found in primates and modularity is organized according to on/off domains (Humphrey et al. 1980a; Lund et al. 1983; Norton et al. 1985; Kretz et al. 1986; Wong-Riley and Norton 1988). Differences have also been reported in the lateral geniculate nucleus (LGN), colliculus and pulvinar between tree shrews and primates (Casagrande et al. 1977; Sherman et al. 1975; Norton et al. 1977; Graham and Casagrande 1986; Lyon et al. 2003). The organization of, and projections to, the LGN and colliculus of tree shrews differ from primates and the clear distinction
between magno- and parvocellular cells observed in the LGN of primates is less conspicuous in tree shrews (for reviews see Casagrande and Norton 1991; Stepniewska 2003). In addition, each colliculus in the tree shrew represents the whole contralateral eye, unlike primates where each colliculus represents only one hemifield (Lane et al. 1971).

Here we found similarities and differences in regional specializations in microstructure in visual cortical areas between the tree shrew and primates. For example, as in primates we found a trend for pyramidal cells with more branched dendritic arbours with progression through areas V1, V2 and a third level cortical area (TD). However, unlike in all other primates studied (marmoset monkey, owl monkey, macaque monkey and the gato-go), there was a trend for smaller cell bodies and smaller dendritic arbours with progression through these areas. In primates, the size of both the cell bodies and the dendritic arbours tends to increase with progression through these areas (Lund et al. 1993; Elston and Rost 1997, 1998, 2000; Elston et al. 1999b, Elston 2003). In addition, comparison of the number of spines in the basal dendritic arbours of pyramidal cells in V1 of these species showed the tree shrew to be very different from marmosets, owl monkeys, macaques and the galápagos shrew; cells in tree shrew V1 were more than twice as sparsus (1,507 spines) as those in the latter species, which as a group were remarkably similar (556-773 spines). However, this finding was not true of all cortical areas. cells in V2 (1,484) and TD (2,531) of the tree shrew have
similar numbers of spines in their basal dendritic arborisations, if not slightly higher, to those reported in V2 and "third level" visual areas in mammals, owl monkeys, macaques and the Galago (Fig. 5). These findings suggest that mechanisms that influence the pyramidal cell phenotype in different cortical areas vary between species.

What then are the possible functional consequences of these differences in pyramidal cell structure? The size of the dendritic arbours of neurons are thought to influence their sampling geometries (i.e., size of the topographic representation from which inputs may be sampled), their branching patterns influence how these inputs are processed within the arbour (e.g., degree of compartmentalization) and the number of spines in the dendritic trees provides an indication of the number of excitatory inputs received by cells (for reviews see Elston 2002; Jacobs and Sheibel 2002). Thus, the present data suggests that, by virtue of their relatively high number of dendritic spines, layer III pyramidal neurons in tree shrew V1 are capable of more complex processing than their counterparts in monkeys (macaque, marmoset, owl monkey) and the galago. That is to say, they are able to compartmentalize a larger number of inputs within their dendritic arbour, allowing greater functional capacity (e.g., Poronat and Mel 2001).

Cells in supragranular V1 typically show selectivity for direction of motion, orientation of an object as well as spectral cues (e.g., Hubel and Wiesel 1968; Humphrey et al. 1980b; Blasdel and Fitzpatrick 1984; Ringach et al. 2002). It is still uncertain whether their orientation and direction selectivity exhibited by these cells stems from an asymmetry of inputs, or an asymmetry in their dendritic trees of recipient neurons (for reviews see Kivisäär et al. 1996; Livingstone 1998; Ferster and Miller 2000). Previously we have demonstrated that V1 cells in the macaque monkey have asymmetrical dendritic arbours relative to those in V2 and other extrastriate visual areas, which may be important for processing orientation and direction selectivity (Elston and Rosa 1997, 1998). However, Martin and White- ridge (1984) found no such systematic correlation in the cat. Here we found that cells in V1 had a greater degree of bias in their dendritic arbours than cells in V2 (mean and standard deviation of aspect ratios 1.115±0.013 and 1.126±0.019, respectively) and TDI (1.198±0.020), suggesting that the structure of their dendritic trees may contribute to their response properties. It remains to be determined whether these differences are attributable to the different methodologies used in the different studies, or whether they reflect species variations. Moreover, a systematic correlation of pyramidal cell structure will shed light on whether orientation/direction selectivity reported in V1 of the tree shrew (Moore et al. 2004) is more exclusively tuned than that reported in macaque monkeys by virtue of their ability to sample a greater number of inputs (i.e., larger number of dendritic spines). Further systematic studies are required in cat, tree shrew and a variety of primates to determine how the relationship among inputs from the lateral geniculate nucleus, projections in V1 from layer IV to supragranular layers, and the morphology of the recipient neurons influences their sensitivity to direction and orientation.

In conclusion, visual cortex in tree shrews shares affinities with both primates and non-primates. Here we demonstrate that regional variation in some aspects of pyramidal cell structure in tree shrew such as branching pattern parallel those reported in primates, whereas others such as the size of the dendritic arbours and cell bodies do not. In particular, layer III pyramidal cells tree shrew V1 differ markedly to those in the galago and monkeys, being more branched and nearly twice as spiny. Further studies of pyramidal cell structure will be necessary, particularly in non-primates, to better understand the evolution and specialization of cortical circuitry.

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