Radial translaminar arrays of pyramidal cells—minicolumns—are a pervasive structural motif of placental mammalian neocortex, which are anticipated in the earliest stages of cortical development by the formation of ontogenetic cell columns comprising radial glial cells and associated radially migrating neurons. In the present study we examine the temporal continuity in these structures throughout development and aging. Computerized image analysis of micrograph Nissl-stained postmortem tissue produced estimates of the median free path through neuropil in the radial direction (parallel to pyramidal cell arrays) and in the tangential direction (parallel to the cortical surface). These data were modeled as a biphasic power law with respect to in utero development and postnatal age, multiplied by a decay factor. No significant change in the ratio of radial to tangential neuropil space was demonstrated in either the prenatal or postnatal sample population. Neuronal development follows a prenatal phase of cubic volumetric growth with a postnatal phase of linear volumetric growth. The data suggest the continuity of columnar structures from early in gestation through postnatal maturation.

Keywords: brain, development, minicolumns, neocortex, neuropil

Introduction

The minicolumn is an elemental modular unit of the cortex (Mountcastle 1997; Buxhoeveden and Casanova 2002) serving as an architectonic template according to which are arranged representative cortical cellular elements and their connections. A chain of excitatory neurons with their axonal and dendritic bundles constitutes its core, extending radially in layers II through VI (Peters and Sethares 1991, 1996). Around the periphery of this core is a neuropil containing synaptic elements integrating translaminar connections. Peripheral neuropil also contains inhibitory interneurons (prominently radially oriented GABAergic double-bouquet cells) providing a "sheath" of inhibitory activity (del Río and DeFelipe 1997). A given minicolumn is characterized functionally by a common set of physiological response properties of its constituent neurons. The basic minicolumnar circuit is distributed iteratively throughout neocortex, incorporated into a nested hierarchy of modular units. Cortical microstructure exhibits variability intra- and interareally across hemispheres within a given brain, as well as among individuals within and across species with regard to cell size, density, number, and size of axonal and dendritic bundles. Nevertheless, the basic organization of microcircuitry is maintained throughout the cortex in all individuals and species (Douglas and Martin 2004; Buxhoeveden and Casanova 2005). Similarly, with changes in connections and synaptic content, temporal as well as spatial contiguity is maintained in minicolumnar organization.

Minicolumnar anatomy has been characterized principally in terms of 4 radially oriented components: pyramidal cell arrays, apical dendritic bundles, myelinated axon bundles of projection neurons, and double-bouquet cell axon bundles of inhibitory interneurons. Each of these features has been shown to exhibit minicolumnar-scale periodicity in various species and cortical areas (von Bonin and Mehler 1971; Seldon 1981; DeFelipe and others 1999; Ong and Carey 1990; Viebahn 1990; Peters and Sethares 1991, 1996; Ferrer and others 1992; del Río and DeFelipe 1997; Peters and others 1997). Peters and Sethares (1996) were the first to characterize the spatial relation of pyramidal cells in layers III and V with the radially oriented translaminar dendritic bundles arising from them, calling these assemblages "pyramidal cell modules." Lohmann and Köppen (1995) further demonstrated in rat V1 cortex that apical dendritic and myelinated axon bundles project in register with each other at minicolumnar-scale intervals (52.6 and 50.1 µm, respectively), consistent with other studies (Buxhoeveden and Casanova 2002). Double-bouquet axons in peripheral neuropil similarly were found to align with myelinated axon bundles. This pattern of alignment and of double-bouquet synaptic contacts with pyramidal cells was found to be similar in human temporal and macaque visual cortex (del Río and DeFelipe 1997). This suggests that they are part of a general organizing motif in primates. Thus, linkages demonstrated among these principal components show that they provide redundant measures of minicolumnar center-to-center spacing and suggest that inferences might be made from measurements related to any component/compartment and overall minicolumnar dimensions. Several studies have endeavored to develop quantitative methods, biased (Schlaug and others 1995; Buldyrev and others 2000; Buxhoeveden and others 2000) or unbiased (Skoglund and others 2004; Vercelli and others 2004) in the stereological sense, for assessing minicolumnar morphology in terms of parameters such as verticality, columnar width, mean cell spacing, compactness, gray-level index, and others.

The developmental relation between ontogenetic cell columns and the mature cortical minicolumn has been heretofore relatively unexplored. Optical imaging of physiologic activity in fetal rat brain slices revealed columnar structure, which the investigators identified with both radial units and mature cortical columnar units (Yuste and others 1992). The diameter of these structures (50–120 µm in neonatal rat) is greater than that typical of minicolumns in mature rat cortex. Further, the linking of cells within them by gap junctions suggests that these structures may be aggregations of cell columns coordinating activity in larger modular units (Weissman and others 2004).
Krmpotić-Nemanić and others (1984) identified vertical cell columns in a limited developmental series derived from human fetal and neonatal auditory cortex, reporting that they could trace the developmental transformation from ontogenetic cell columns into mature minicolumns. A later study of human fetal cortical development identified lamina-specific differences in emergence of minicolumnar morphological features (Buxhoeveden and others 1996). McKinstry and others (2002) conducted an in vivo imaging study of human cortex in subjects aged 26–40 gestational weeks (GW). They employed diffusion tensor imaging to determine patterns of water diffusion anisotropy in developing cortex. Water diffusion patterns are determined by tissue microstructure and reflect alignment of lipophilic cell membranes and myelin sheaths. Radially oriented anisotropy at 26 GW indicates vertical orientation of cortical structures. At 36 GW radial anisotropy decreases, reflecting the superimposition of tangentially oriented collateral elements upon the primary vertically oriented cortical structure. None of these previous studies, however, employed quantitative methods to determine contiguity of radial structures during development.

The purpose of our study was to provide an unbiased quantitative analysis of change over time in the morphometry of minicolumnar peripheral neuropil. We derived a model to describe rate of change in median neuropil space (MNS) or average radial or tangential distance from a given point in neuropil space to a threshold boundary delimiting a cellular compartment. A ratio of radial to tangential MNS greater than unity is consistent with vertical orientation of peripheral neuropil space. Lack of significant change in the ratio of radial to tangential MNS during gestation and postnatal life is consistent with a stable vertical columnar organization. This is consistent with a stable vertical columnar organization. This position of these textures is reflected in J where minicolumnar structure stands out as a strongly directional signal against an isotropic background. The dominant orientation $\theta$ of the signal is found using the formula (Pourdeyhimi and others 1994)

$$\theta = \frac{1}{2} \tan^{-1} \left( \frac{\sum J_k \sin 2\theta_k}{\sum J_k \cos 2\theta_k} \right),$$

where $|J_k|$ is the amplitude of component $k$ of the discrete Fourier transform, and the sum is over all 2-dimensional frequencies $\mathbf{k} = (k_x, k_y)$. The angle $\theta_k$ is not the phase of $J_k$ but the direction of the signal giving rise to the Fourier component at that frequency, that is $\tan \theta_k = g_k / f_k$. The directionality, or strength of the directed texture, is given by Pourdeyhimi and others (1994) as

$$I^2 = \sum_k |J_k|^2 \cos^2 (\theta_k - \theta),$$

which is normalized to lie between zero and one.

A local estimate of $r^*$ is produced by computing the above quantities in small (about 0.20 mm$^2$) regions of an image:

$$r^*_{\text{region}} = (\cos \theta_{\text{region}}, \sin \theta_{\text{region}}).$$

Two modifications to this basic method ensure that the estimates of $r^*$ do not vary unrealistically across the image: neighboring regions are made to overlap substantially so that the respective estimates therein are not independent. In addition, the final result is smoothed by a 3-by-3 weighted median filter. Local directionalities $I^2$ are used for weights, and the median filter is applied to the components of $r^*$ individually.

The neuropil distribution is derived from the empirical “linear contact distribution” (Fig. 1), the distribution of distances from pixels in the region of interest to the nearest object, measured along a line with fixed orientation $\varphi$. In the event that the pixel of origin is itself part of an object, the nearest neighbor distances are zero. Let $L(r, \varphi)$ be the cumulative distribution function of the linear contact distances, and let $\tilde{L}(r, \varphi) = L(r, \varphi) / L(0)$ be the cumulative distribution function conditioning on the event that the pixel of origin lies in the neuropil

$$\tilde{L}(r, \varphi) = \frac{L(r, \varphi) - L(0)}{1 - L(0)}.$$

Then the median radial neuropil space is $s_0 = \tilde{L}^{-1}(0.5; 0)$, and the median tangential neuropil space is $s_\varphi = \tilde{L}^{-1}(0.5; 0 + (\pi/2))$.

### Results

MNS estimates were binned for purposes of statistical analysis. The mean of $s_0$ (respectively, $s_\varphi$) over the 3 or 4 micrographs of each brain was taken as the estimate of $s_0$ (respectively, $s_\varphi$) for the temporal/parietal region of the cerebral cortex. Postnatal neuropil development was modeled as a power law, representing early growth, and multiplied by an exponential curve, representing the effects of aging. Prenatal development was modeled to follow a power law with a different growth rate. Although the growth rate may change at birth, the neuropil space must change continuously with age: prenatal and postnatal developmental function curves are contiguous. These considerations lead to the functional form $s = b_0 g^b (g + a)^b_c e^{-b_c a}$.
where $g$ is gestational age divided by 40 weeks and $a$ is postnatal survival, also divided by 40 weeks. The dependent variable $s$ is the MNS (radial or tangential) for a given individual.

The model was fit (Table 1) using ordinary least squares after taking the logarithm of both sides. Parameters $b_0$, $b_1$, and $b_2$ were functions of direction (tangential or radial), and $b_0$ was modeled as a function of the sex of the individual and direction. Each of the 4 parameters differed significantly ($P < 0.05$) from zero with Student $t$ scores 65.2, 8.8, 12.8, and 5.1 for $b_0$, $b_1$, $b_1'$, and $b_2$, respectively, with 59 degrees of freedom. Section thickness (see Materials) was not accounted for, so neuropil in the youngest prenatal cases may have been overestimated. The effect on the results, if any, would be a bias toward zero in $b_1$. Directional dependence was significant only for the parameter $b_0$ ($t = 3.02$). Sex dependence in $b_0$ was statistically insignificant ($t = -0.16; P = 0.87$). Significance tests on $t$ scores were 2 sided.

Statistically significant directional dependency of the neuropil space, with the median distance measured radially exceeding the median distance measured vertically, is consistent with a columnar arrangement. Here, the bulk of the neuropil lies in the periphery of minicolumns, where the mean free path is greater in the radial direction, staying within the periphery, than in the tangential direction, oriented toward a pyramidal cell array. There is no evidence that the ratio of median radial neuropil space to median tangential neuropil space changes over time (Fig. 2). The absence of significant directional dependency in the rates of change in neuropil space with aging suggests that the basic columnar structure is preserved throughout pre- and postnatal development.

The estimated rate of prenatal neuropil growth is $b_1 + b_1' \approx 1$. Because MNS $s$ has dimensions of length, the total volume of cortical neuropil increases “cubically” with time in utero. Shortly after birth, the estimated growth rate drops to

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**Figure 1.** Estimation of the linear contact distributions. The local radial direction $r'$ in each panel is $30^\circ$ anticlockwise from vertical. The free path from a point in the neuropil (center of red circle) is the distance to the nearest object in either direction along a straight-line path with fixed orientation. Typically, the radial free path is greater than that in the tangential direction (A), although the opposite also obtains (B). The free path is identically zero for points outside of the neuropil (C). The free path from points near the boundary of the field of view (D) may be less than the distance to the nearest visible object; the data are censored from above. Such observations are incorporated using a Kaplan-Meier-type estimator for the linear contact distribution.

**Table 1**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>MLE</th>
<th>CI</th>
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<tbody>
<tr>
<td>$b_0$</td>
<td>7.69 μm</td>
<td>(7.08 μm, 8.36 μm)</td>
</tr>
<tr>
<td>$b_1$</td>
<td>6.43 μm</td>
<td>(5.92 μm, 6.98 μm)</td>
</tr>
<tr>
<td>$b_0'$</td>
<td>0.62</td>
<td>(0.48, 0.76)</td>
</tr>
<tr>
<td>$b_1'$</td>
<td>0.33</td>
<td>(0.28, 0.38)</td>
</tr>
<tr>
<td>$b_2$</td>
<td>0.0064</td>
<td>(0.0039, 0.0088)</td>
</tr>
</tbody>
</table>

Note: Insignificant effects are averaged, so that values of $b_0$ are the average for male and female, and values of the other parameters are the average over both directions.

MLE = maximum likelihood estimate; CI = 95% confidence interval; rad = radial direction; tan = tangential direction.
b_1 \approx 1/3$, corresponding to cortical neuropil volume increasing “linearly” with age. The effective rate of postnatal neuropil growth decreases due to the decay term $b_2$ and becomes zero at about 40 years of age and negative thereafter.

The model was subsequently fit to the postnatal series alone in order to examine possible differences between cortical areas (Fig. 3). Parameter $b_1$ was omitted because $g$ is assumed equal to unity for the postnatal series. Parameters $b_0$, $b_1$, and $b_2$ were permitted to vary with cortical area. No statistically significant difference in $b_0$ was found between areas ($F_{3,142} = 0.564$, $P = 0.64$). Regional variability in $b_1$ and $b_2$ were even less significant.

**Discussion**

Our study is the first to employ unbiased quantitative morphometric techniques in the analysis of the development of radial structure in cortex. Our principal finding provides strong support for the hypothesis that values of structural parameters of neuropil space exhibit stability over the course of development. Our findings suggest that continuity is maintained in the morphometry of radial minicolumns in human neocortex throughout the life span, from emergence of the laminated cortical plate until late maturity. Additionally, our data were derived from postmortem tissue exhibiting no sign of neuropathology and obtained from individuals dying from nonneurological causes. Our results thus provide normative data as a basis for comparison analysis of minicolumnar morphometric changes potentially associated with various neurological or psychiatric pathologies.

The minicolumnar organization of cortex is anticipated in the earliest stages of telencephalic development. The ventricular neuropil represents a mosaic of radial units. These are spatially discrete clonal aggregates consisting of neuronal precursors and transient radial glial (RG) cells that serve as progenitors for their radial units. Neurons thus migrate radially outward into the cortical plate along the processes of RG cells. Glial processes provide a lattice for maintaining the linear spatial organization of daughter neurons, which in later stages migrate hundreds of microns into an expanding cortical plate that is starting to form convolutions and convexities (Rakic 1988; Kriegstein and Noctor 2004). These ontogenetic cell columns are the first vertically oriented structures appreciable in the fetal cortex and provide the matrix for its further development. Subsequently, neurites grow radially from the soma of ontogenetic columns and develop first into apical dendrites and then other processes (Hirst and others 1991). Ontogenetic cell columns have been identified in a wide range of mammalian species, suggesting that they are a well-conserved feature of cortical development (Gressens and Evrard 1993).

Selective influences operating during development and on functional circuits in the mature cortex combine to maintain continuity of radial morphology throughout the life span. Phylogenetic expansion of the mammalian cortical sheet, particularly in primates, required adaptations of radial units of glia and their related neuronal progenitors. Increased gyrification in larger primate cortices resulted in longer, curvilinear radial migratory pathways (Rakic 2003). Associated with this trend, RG and neuronal progenitor populations in ventricular zone exhibit specialized molecular and structural phenotypes (Hartfuss and others 2001; Rakic 2003; Weissman and others 2003). These features are identified at the earliest stages of corticogenesis and in primate radial glia include early glial fibrillary acidic (GFAP) immunoreactivity and lamellate expansions (Schmechel and Rakic 1979; Levitt and Rakic 1980; Levitt and others 1981, 1983). This neocortical expansion provided the substrate for functional specialization of minicolumns derived from these radial units, both locally within macrocolumnar aggregates, as well as among various cortical areas. Regional differences in proliferative kinetics, both of symmetrically dividing clonal founding progenitors as well as progenitors within each clonal unit could result in regional variation in minicolumnar numbers and size (Kornack and Rakic 1998). Hypothetically, each of a diverse array of specialized minicolumns could provide a structurally stable template upon which plastic and specific activity-driven circuits could later be imposed.

A notable feature of the enlarged primate cortex and its regional and columnar specializations is the large proportion of inhibitory interneurons derived from dorsal telencephalic proliferative zones (Lentinic and others 2002) in contrast to rodent cortex in which most interneurons originate in the subpallium (Marín and Rubenstein 2003). The primate brain contains a greater proportion and diversity of interneurons (DeFelice and others 2002). Radially migrating lineages of inhibitory interneurons on the basis of common intrinsic mechanisms and signaling environments may be better coordinated with colocalized pyramidal cells in the formation of minicolumns exhibiting greater numbers and types of interneurons. Regional- and laminar-specific production of inhibitory and excitatory neurons might thus be closely matched. This is particularly relevant to the development of layer IV, as differences in total number of neurons in columnar volume between various cortical areas are due largely to differences in numbers of layer IV cells (Beaulieu and Colonnier 1989).

This is also relevant to the greater elaboration and plasticity of inhibitory synaptic networks in layer IV. In rats, layer IV had 62% more GABAergic synapses than other layers due to increased number of contacts per bouton, rather than higher number of boutons, with a comparable total area of synaptic coverage (Beaulieu and others 1994). Remodeling of synaptic elements occurs within a framework of axonal and dendritic arbor and boutons that maintains morphometric stability. An enriched environment for rats results in consolidation of the number of
synaptic contacts in visual cortex layer IV but does not affect the ratio of boutons per neuron (Beaulieu and Colonnier 1988). Stable laminar-specific neuronal architecture allows for compartmentalization of function within each layer. Mean densities as well as the ratio of symmetric to asymmetric synaptic contacts in macaque V1 are not affected by early enucleation. Ratio of spine-to-shaft contacts in layer IV does not undergo comparable shift seen in animals with normal vision. Thus, experimental animals exhibit layer-specific (to thalamoreceptive sublayers) alteration of fine-scale contacts within minicolumns whose overall morphometry is preserved (Bourgeois and Rakic 1996). As well as increased numbers of inhibitory interneuron elements within primate striate cortex layer IV, the developing primate cortical mantle also exhibits an expanded subplate (Kostović and Rakic 1990). Transient radial circuits between subplate and layer IV subserve the establishment and organization of thalamocortical projections to layer IV and subsequent synaptic remodeling of those projections (Allendoerfer and Shatz 1994; Kanold 2004). Ablation of subplate affects organization of both ocular dominance columns (Ghosh and Shatz 1992) as well as minicolumnar-scale orientation columns (Kanold and others 2003).

Minicolumnar compartmentalization may also be affected by geometric constraints, which serve to maintain overall morphometry. Layer II neuron counts are comparable across cat visual areas. Whereas the monocular region of area 17, area 18, and posteromedial suprasylvian have comparable cell counts under a specified area (Rockel and others 1980), the laminar distribution of neurons varies. Thus the relative decrease in cell numbers in layer IV of the latter 2 areas is compensated for by increases in other layers, primarily layer III (Beaulieu and Colonnier 1985). This finding suggests that the total cell count of the minicolumnar laminae and corresponding minicolumnar dimensions might be constrained by packing limits imposed by their associated radially oriented processes. Thus, greater width of axonal and apical dendritic bundles and of vertically oriented inhibitory interneurons required to modulate them would increase the tangential distance between minicolumns requiring ever greater expansion of the cortical sheet. This subsequently would impose escalating wiring, metabolic, and signal-timing costs (Striedter 2005). Neocortical minicolumns therefore serve as a modular template or tabula rasa within which a stable matrix of dendritic and axonal arbors provides an array of potential synaptic connection sites (Kalischen and others 2005). These sites develop into functional synaptic connections presumably via coordinated pre- and postsynaptic activity. Multiple functional microcircuits exhibiting both fine-scale specificity and plasticity can be overlain within this common matrix of potential connections (Stepanyants and Chklovskii 2005).

The maintenance of constant ratios of radial to tangential MNS over the course of development and the scale of these dimensions on the order of 10 μm suggests that these morphometric relations reflect an intrinsic circuit organization present from the earliest stages of cortical plate formation. This fundamental organization of neuropil may serve as a template upon which later epigenetic influences operate. Ben-Ari and others (2004) reviewed evidence obtained in hippocampus indicating that GABAergic interneuron networks play a formative role in the organization of cortical microcircuits. They suggest that early—initially excitatory—GABAergic synapses among phenotypically diverse interneurons in peripheral neuropil establish patterns of activity by which pyramidal neurons, quiescent at this stage, may later be coordinated in the formation of circuits. As robust radially oriented morphometry implies stereotypical synaptic organization, so conversely,
stereotyped arrangements of cells and their connections into radially oriented circuits implies an origin in early ontogeny for radial orientation of cortical structure. Badd and Kisvárday (2001) and Kozloski and others (2001) showed that local connections are highly specific, stereotypic, and determined, tending to fall within minicolumn dimensions. Kozloski and others (2001) found precisely determined cortical microcircuits in mouse layer V cortex, showing that local connectivity is not probabilistic or random. The position of the targeted neurons appeared “remarkably precise, indicating robust developmental control of circuit formation.” Stability of minicolumnar architecture throughout the life span supports recent findings that neuronal generation in the mature brain is limited to specialized niches (Kornack and Rakic 2001). Introduction of newborn neurons in the adult cortex might result in heterotopic structures potentially disrupting the organization of established specific circuits.

This high degree of developmental continuity in the radial alignment of peripheral neuropil spatial features may in part reflect an optimization of the density and spatial arrangement of processes and synaptic elements in relation to neuronal cell bodies. This optimization may encompass constraints of signal timing, metabolism, and degree of dendritic arborization required to maintain given synaptic densities, all in turn determining cell body packing arrangements (Rakic and others 1994; Chklovskii and others 2002; DeFelipe and others 2002). In support of this view, a number of investigators as cited by DeFelipe and others (2002) have reported a degree of uniformity in synaptic densities among different cortical areas within and across species (O’Kusky and Colonnier 1982; Beaulieu and Colonnier 1989). This uniformity is anticipated in a common time course and rate of production/regression of synapses observed throughout primate cortex (Rakic and others 1986). Subsequently, other studies using more refined quantitative methods revealed significant differences in neuronal and synaptic density across layers within a given area in intraspecies and cross-species comparisons of visual and somatosensory cortex in mouse, rat, monkey, and human (Beaulieu and others 1992, 1994; DeFelipe and others 2002). These differences may reflect a functional laminar compartmentalization of minicolumnar architecture whereby synaptic density is regulated by the interaction of cell-intrinsic determinants with thalamocortical and monoaminergic modulatory inputs. For example, dopamine D1 and D2 receptors have a layer- and region-specific distribution (Lidow and others 1991) and modulate synapses of projection neurons (Yang and Seamans 1996). This activity serves to gate corticocortical and corticothalamic feedback activity underlaying working memory (Sawaguchi and Goldman-Rakic 1991) and is reflected in regulation of synaptic densities in prefrontal cortex (Sugahara and Shiraishi 1999).

A number of investigators have asserted a general inverse relation between neuronal density and number of synapses per neuron (Cragg 1967; O’Kusky and Colonnier 1982; DeFelipe and others 2002). In analyses of human, rat, and mouse cortex this relation was confirmed in comparing all layers of human cortex with those of rat and mouse, yet the relation did not hold in interlaminar comparisons in human and mouse (DeFelipe and others 2002), nor was there a common pattern found in determining ratios of asymmetric to symmetric synapses. Notably, this group found that thickness of layers II and III in the human was greater than twice that of the rat and 3 times that of the mouse, whereas the neuronal density was less than one-half and one-quarter of that of the rat and mouse, respectively. This plainly suggests the presence of nonneuronal components and a spatial organization specific to supragranular layers of human cortex. Human cortex contains a greatly expanded network of astrocytes in comparison with nonprimate species and, in particular, palisades of vellate astrocytes with superficially situated cell bodies, and processes extending inward through supragranular layers may represent a significant nonneuronal component of neuropil (Reisin and Colombo 2002; Colombo and Reisin 2004). It will thus be profitable for future studies to conduct similar morphometric analyses of verticality in neuropil using immunocytochemical labeling for GFAP or other nonneuronal markers.

In summary, the results of this study are notable for the fact that no significant change in the ratio of radial-to-tangential neuropil space was demonstrated in 67 individuals representing a full range of prenatal and postnatal stages of cortical development. This lends strong support to the hypothesis that there is a fundamental temporal continuity in the vertical organization of cortex, preeminent to formation of laminae and other higher-order organization, for example, synapse formation. This vertical organization provides the fundamental structural motif within which processes mediating cortical plasticity such as synaptic remodeling and apoptosis might occur during the course of development.


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