AGE-RELATED REDUCTION IN MICROCOLUMNAR STRUCTURE CORRELATES WITH COGNITIVE DECLINE IN VENTRAL BUT NOT DORSAL AREA 46 OF THE RHESUS MONKEY


Abstract—The age-related decline in cognitive function that is observed in normal aging monkeys and humans occurs without significant loss of cortical neurons. This suggests that cognitive impairment results from subtle, sub-lethal changes in the cortex. Recently, changes in the structural coherence in mini- or microcolumns without loss of neurons have been linked to loss of function. Here we use a density map method to quantify microcolumnar structure in both banks of the sulcus principalis (prefrontal cortical area 46) of 16 (ventral) and 19 (dorsal) behaviorally intact female rhesus monkeys from 6 to 33 years of age. While total neuronal density does not change with age in either of these banks, there is a significant age-related reduction in the strength of microcolumns in both regions on the order of 40%. This likely reflects a subtle but definite loss of organization in the structure of the cortical microcolumn. The reduction in strength in ventral area 46 correlates with cognitive impairments in learning and memory while the reduction in dorsal area 46 does not. This result is congruent with published data attributing cognitive functions to ventral area 46 that are similar to our particular cognitive battery which does not optimally tap cognitive functions attributed to dorsal area 46. While the exact mechanisms underlying this loss of microcolumnar organization remain to be determined, it is plausible that they reflect age-related alterations in dendritic and/or axonal organization which alter connectivity and may contribute to age-related declines in cognitive performance. © 2009 IBRO. Published by Elsevier Ltd. All rights reserved.

Key words: aging, cognition, minicolumns, microcolumns, neuronal organization, primate brain.

While conventional wisdom posits that age-related cognitive decline is the result of age-related neuronal loss, this notion has not stood up to experimental scrutiny. Recent studies have asserted that cognitive decline in normal aging, in contrast to other age-related neurodegenerative disorders such as Alzheimer's disease, is not accompanied by significant loss of neurons in the cerebral cortex (e.g. Pakkenberg and Gundersen, 1997; Peters et al., 1998; Pakkenberg et al., 2003). Considerable evidence has instead implicated other brain changes including changes in synapses, dendrites, and white matter that would likely after cortical information processing and may therefore better account for age-related cognitive decline (e.g. Peters and Rose, 2003). This evidence does not rule out other subtle changes that might be occurring in gray matter and that may equally well contribute to cortical dysfunction. One important feature of the cortex, and a possibly vulnerable element, is the vertical arrays of neurons called minicolumns. Minicolumns, or "microwolumns" as referred to in this paper, are usually found spanning from the subcortical white matter to the pial surface (Mountcastle, 1997, 2003). Microcolumns sometimes share terminology with other possibly related intracortical elements such as bundles of apical dendrites (Peters, 1994; Rockland and Ichimochi, 2004) or axons of pyramidal cells and pyramidal cell modules (DeFelipe et al., 1990; Jones, 2000). Following the early physiological studies by Mountcastle (1957) and Hubel and Wiesel (1963, 1969, 1977) on the much larger macrocolumns, microcolumns have been characterized as a fundamental unit of organization and function (Mountcastle, 1997, 2003; Peters and Sathares, 1996; Constantinidis et al., 2001; Buchel and Casanova, 2002; Vercelli et al., 2004). This characterization, however, is less universally accepted as numerous critiques question the validity of the microcolumn as a fundamental unit of organization or function (Swindale, 1990; Purves et al., 1992; Jones, 2000; Rockland and Ichimochi, 2004; Buchel and Casanova, 2005; Krieger et al., 2007). Other studies of "whisker columns" or barrels in the rodent somatosensory cortex (the anatomical equivalent of a functional cortical column) indicate that these columns are not a functional unit consisting of cells with similar functional properties (Heilmaa et al., 2007). Even further, others argue that the very well established and much studied ocular dominance columns seem not to serve a purpose (Horton and Adams, 2005).

Independent of the exact function of the microcolumn, a growing body of studies has reported structural changes in microcolumns that are related to a general loss of cognitive function in normal aging and in various disease states. These studies have dealt with differences in neuron distribution in general, and within microcolumns in particular, and span topics from normal aging (Cruz et al., 2004),
Alzheimer's disease and Lewy body dementia (Van Hoesen and Sokolovkin, 1993; Bulychev et al., 2000), schizophrenia (Benes and Bird, 1997; Bushuevden et al., 2000), Down syndrome (Bushuevden et al., 2002), autism (Casanova et al., 2003), dyslexia (Casanova et al., 2002), to drug (cocaine) manipulation during development (Bushuevden et al., 2006). Interestingly, while we have noted that the number of cortical neurons is largely preserved in normal aging monkeys, evidence of age-related physiological disruption of orientation selective microcolumns has been reported (Schmolesky et al., 2000; Leventhal et al., 2003). These investigators reported a loss of orientation and direction selectivity in microcolumns in V1 and demonstrated that administration of GABA agonists temporarily restored normal stimulus selectivity to these neurons. Since small GABAergic interneurons are an important, though poorly understood, component of the microcolumn (Mountcastle, 1997) this suggests that there may well be a disruption within the microcolumn as part of normal aging.

We previously reported a 40% loss of microcolumnar strength in area 16 of the ventral bank of sulcus principalis in aging monkeys and determined that this loss of strength correlates with cognitive decline (Cruz et al., 2004). In the present study, we examine age-related changes in microcolumns in tissue from a larger cohort of normal aging monkeys and compare microcolumnar integrity in area 16 of the prefrontal cortex in the upper (dorsal) and lower (ventral) banks of the mid level of sulcus principalis. In terms of function, damage to the lateral prefrontal cortex, including areas 9, 46, and 9/46, is well-known to cause deficits in performance of tasks requiring executive functions, noting that this broad area also takes part in operations that include abstraction, response selection, working memory operations, memory encoding and retrieval. Data from humans and monkeys, as reviewed by Petrides (2005), suggest that for the midlateral prefrontal cortices that we examine, ventral area 46 is likely involved in processing information in conjunction with the posterior parietal, temporal, and occipital association cortices for both the encoding and retrieval aspects of memory processes. In contrast, dorsal area 46 is likely involved in monitoring working memory operations important for processes such as abstraction or set shifting.

To quantify the microcolumnar structure of these two areas we apply a density map analysis (Cruz et al., 2005) to images from ventral 46 (46V) and dorsal 46 (46D) from a cohort of 19 normal aging female rhesus monkeys. The goal of this comparison is to determine if the previously observed loss of microcolumnar strength reported in a smaller cohort for 46V (Cruz et al., 2004) is localized to 46V or a more global age-related effect, and to explore the possibility that changes in these areas are related to their specific cognitive functions.

**EXPERIMENTAL PROCEDURES**

**Subjects**

Brain tissue was obtained from 19 female rhesus monkeys ranging from 6.4–12.9 years of age. This material was derived from archived tissue samples available from a study of the neural bases of age-related cognitive decline. Hence no animals were utilized explicitly for this study. Under the procedures of that study, animals were selected from the population of monkeys at the Yale University Research Center (YNPRC) at Emory University according to strict selection criteria that excluded any monkeys with health or experimental histories that could have affected the brain or cognitive function. The lifespan of the rhesus monkey compared with the human corresponds approximately to a ratio of 1:3 (Tigges et al., 1988). Monkeys therefore can be considered young adults at age 5 when they reach sexual maturity, which would roughly correspond to a 15-year-old human. Few monkeys live beyond 30 years of age, which would roughly correspond to humans over 50 years old (Tigges et al., 1988; Roth et al., 2004).

**Animal procedures**

After entering the study monkeys were first maintained at the YNPRC, and subsequently at the Laboratory Animal Science Center of the Boston University Medical Center. Both facilities are fully accredited by the Association for the Assessment and Accreditation of Laboratory Animal Care. All procedures were approved by the Institutional Animal Care and Use Committee of both Emory University and Boston University Medical Campus, and conformed to the NIH Guide for the Care and Use of Laboratory Animals and the U.S. Public Health Service Policy on Humane Care and Use of Laboratory Animals. Monkeys received initial Magnetic Resonance Imaging (MRI) scans (Intra 3T Scanner, Philips Instruments, North Andover, MA, USA) to ensure there was no occult pathology in the brain and then were tested on a battery of cognitive tests to characterize learning and memory (e.g. Hemdon et al., 1997) and executive function (Moore et al., 2005). At the conclusion, monkeys were tranquilized with ketamine hydrochloride, deeply anesthetized with sodium pentobarbital, and killed by exsanguination during transcardiac perfusion of the brain with 4–8 l of warm (37°C) fixative containing either 4% paraformaldehyde alone or a mixture of 1% paraformaldehyde and 1.25% glutaraldehyde in 0.1 M phosphate buffer (pH 7.4). Immediately after perfusion fixation of the brain, the brain was blocked, in situ, in the coronal stereotactic plane. One hemisphere was then cryoprotected in graded solutions culminating with 25% glycerol and 2% DMSO in phosphate buffer (0.1 M, pH 7.4) after which the blocks were flash frozen by immersion in −75°C Freon (Rosene et al., 1996). All blocks were stored at −80°C until cut.

**Tissue processing**

Frozen blocks were oriented on the freezing stage of a slide microtome and frozen sections cut in the coronal stereotactic plane into eight interrupted series of 30 μm thick sections and one series of 60 μm thick sections so that sections in each series were spaced 300 μm apart. One 30 μm thick series and the 60 μm thick series were mounted on subbed microscope slides, allowed to dry overnight and then stained for Nissi substance with Thionin, dehydrated, cleared, and coverslipped. This cryoprotection and cutting procedure produced minimal shrinkage or distortion so that (x,y,z) dimensions were largely preserved through the cutting procedure (Rosene et al., 1996). Once mounted, the sections adhered rapidly to the subbed surface of the slide before they dried, preserving the x-y relationships within the section which was a requisite for all of our analyses described below. However, as sections dried and were subsequently stained, dehydrated and coverslipped, they shrank in the z dimension (thickness) from the originally cut thickness of 30 μm to an average measured section thickness of 7–10 μm, as determined using the Bioquant Image Analysis system (Bioquant Inc, Nashville, TN, USA) equipped with a Heldarheim stage micrometer that measured z axis position to 0.1 μm. Hence the analysis of neuronal locations was limited to this collapsed x-y projection as discussed below.
Cytoarchitectonic regions to be studied

Area 46 is a neocortical subdivision of the prefrontal cortex that is implicated in working memory and executive functions (Goldman and Rosvold, 1970; Goldman et al., 1971; Goldman-Rakic, 1988; Petrides, 2000a,b; Moore et al., 2002a,b). Area 46 in the upper and lower bank of the middle part of sulcus principalis was selected for study, where in coronal sections the cortex is relatively flat (Fig. 1a and b). Our analysis was limited to layer 3 of area 46 for several reasons. First, layer 3 is the area where the majority of cortico-cortical projections that subserve cortical information transfer originate and where the cortico-cortical projecting pyramidal cells have been most carefully studied (e.g. Page et al., 2002; Duan et al., 2003; Chang et al., 2006) making this layer particularly pertinent to the cortical cognitive functions that change with age. Second, as shown in Fig. 1c and d, neurons in layer 3 are relatively loosely packed compared with the smaller, more tightly packed neurons found in adjacent layers 2 and 4. This fact facilitated the reliable identification of neurons, a requirement for our quantitative analysis. Third, because microcolumns span from layer 6 to layer 2 and spacing and other features vary widely across layers, a composite average from all layers would obscure real features. Since analysis of all of the layers of cortex was beyond the scope of our technical capacity, we chose to limit this investigation to layer 3 for the reasons cited as well as to follow up on our previous investigation (Cruz et al., 2004). Due to this restriction, however, we cannot yet assess whether columnarity in other layers is affected by age, or how they may relate to cognitive functions.

Digital photography

Two 30 µm thick Nissl-stained sections per case spaced 300–600 µm apart from area 46 in the middle third of the sulcus principalis were selected (Fig. 1a and b) according to the architectonic criteria of Petrides and Pandya (1994). The levels were matched across all cases. A series of photomicrographs from both banks of sulcus principalis was digitized from both sections with a

Fig. 1. (a) A coronal section of the frontal lobe at the level of the sulcus principalis, area 46, where photographs were digitized (scale=5 mm). (b) A higher magnification of the boxed area from (a) with two boxes delineating areas of layer 3 from the ventral (bottom) and dorsal (top) banks of the sulcus illustrating the location of layer 3 (scale=1 mm). (c, d) Higher magnification photomicrographs of the boxes in (b) illustrating typical images that were analyzed using the density map method. (c) Ventral and (d) dorsal bank of the sulcus principalis (scale bar=100 µm for both panels).
digital camera at 1280×1024 resolution using a 10× objective on a Nikon E600 microscope (Nikon Inc, Melville, NY, USA). These photomicrographs spanned from the bottom of layer 1 through the top of layer 4 and were then cropped such that a square central area of resolution 512×512 pixels was entirely inside layer 3 (Fig. 1c and d). In each bank of the sulcus, four adjacent (non-overlapping) 10× images of layer 3 were acquired. Due to differences in acquisition equipment used, there were slight differences in digital magnification across subjects, where all images for each subject had areas of either 320 μm×320 μm (1.6 pixels per μm), 332 μm×332 μm (1.54 pixels per μm), or 341 μm×341 μm (1.5 pixels per μm). Images from dorsal area 46 from all 19 monkeys were acquired, but due to tissue artifacts images from ventral area 46 in only 16 of the 19 monkeys were acquired. Also, small regions in the images containing tissue folds, large blood vessels, or other artifacts were computationally excluded to avoid including areas of the tissue from which no neuronal data were obtained. In total, eight images (four photomicrographs×two sections of area 46) from each of 19 subjects in dorsal area 46 and from 16 of those subjects in ventral area 46 were analyzed for a total of 280 images.

Identification of $x,y$ coordinates of neurons

To locate all of the neurons in every image, a semi-automatic method of neuron detection was first applied (Cruz et al., 2005) to get the majority of the neuronal locations with an accuracy of about 80–85% but with the disadvantage of obtaining about 15–20% false positives. Using an off-the-shelf image editing program, all of these neuronal markings were then manually corrected to eliminate the false positives and to add the missed neurons in every image after which the final $(x,y)$ coordinates for detected neurons were recorded. All of these $(x,y)$ coordinates were independently corroborated using our newly developed automated neuron recognition algorithm (ANRA) (Ingle et al., 2008) optimized for layer 3 of the cortex.

Density map method and microcolumnar quantities

Microcolumns are vertical arrays of neurons with dimensions in the micrometer scale (Jones, 2000). Fig. 2 shows an example of neurons that line up in a fashion typical of layer 3 microcolumns. To characterize the degree of microcolumnarity of a region of tissue, we developed the density map method that can be used to quantitatively assess and compare different regions in terms of degree of microcolumnarity. This density map method was initially presented by Budayev et al. (2000) and a more detailed quantification and validation was given in Cruz et al. (2005). Briefly, the density map method calculates the density correlation function $g(x,y)$ using as input the $(x,y)$ neuronal coordinates from an image. This function $g(x,y)$ can be mapped to a two-dimensional grayscale image in which different shades of gray are proportional to the average neuronal density relative to the location of each neuron in the region of interest (ROI). This quantity represents the local neuronal density at a particular location $(x,y)$ as opposed to the global neuronal density that specifies the sample-wide average neuronal density with a single number. Thus, the density map represents the average neuronal neighborhood (local) surrounding a typical neuron within a larger ROI.

Operationally, the density map is calculated by first assigning indices $(i=1,2,3,...,N)$ to all the neurons in the sample. Next, we define an origin outside of the sample (Fig. 3a) and create a copy of the locations for the neuron labeled $1$ and shift this copy so that neuron $1$ overlays the origin (Fig. 3b). This process is repeated for each of the detected neurons (Fig. 3c) until an overlay of $N$ copies of the locations in the sample is obtained (Fig. 3d, right image). Over this overlay we then center a grid of bins of size $D$ (Fig. 3d) and count how many neurons fall in each bin, thus constructing one matrix (from the grid of) accumulated neurons $m(x,y)$ for the sample. We define $g(x,y) = m(x,y)/N^2$, in which $g(x,y)$ has units of an average density of objects at position $(x,y)$. This density func-

Fig. 2. Photomicrograph illustrating a column of cells (indicated by the box) that are likely part of a microcolumn and are typical of a "columnar" cortex (scale bar=100 μm).

tion would be uniform if locations of objects (neurons) are uncorrelated, but will show patterns when there are any spatial correlations between the objects. For the case of neurons forming microcolumns in the cortex, their density map exhibits one central vertical ridge, sometimes accompanied by two less pronounced parallel neighboring ridges (Fig. 3d).

From the resulting density map, we quantify the microcolumnar structure by extracting the following measures (Fig. 3e):

- $S$, strength of microcolumns (dimensionless ratio of the neuronal density within a microcolumn to the average neuronal density of the ROI),
- $T$, degree of microcolumnar periodicity (dimensionless ratio of the neuronal density of neighboring microcolumns to the average neuronal density),
- $W$, microcolumn width,
- $P$, distance between microcolumns,
- $L$, length (vertical span) of microcolumns,
- $Y$, distance between neurons within a microcolumn (perpendicular to pia and parallel to microcolumns).

In this study, one density map per monkey per area (dorsal or ventral) was calculated using for each area the eight images acquired as described above (see Digital photography section in Experimental Procedures above). From each density map the six microcolumnar properties plus the neuronal density were then calculated. Thus, for each subject two sets of microcolumnar properties were obtained, one for each area (dorsal and ventral).

Behavioral assessment of young adult and aging rhesus monkeys

Monkeys were given a battery of behavioral tests known to be sensitive to age-related cognitive impairments (Hamond et al., 1997). These tests included the Delayed Non-matching to Sample test (DNMS), both its acquisition (learning) at a 10 s delay (DNMS-10) and DNMS performance (recognition memory) at longer delays (2 min delay, DNMS-120), and the Delayed Recognition Span Task (DRST) which is a test of working memory span and is given in both
spatial and object modalities. We also calculated the Cognitive Impairment Index (CII) which is a composite average of the impairments in z-scores relative to young adults of performance on DNMS-10, DNMS-120, and DRST spatial. The details of these are described in Hendon et al. (1997). The tests are administered in the order listed and are all positively rewarded. For a variety of reasons not all monkeys completed all tests. Studies in monkeys and humans suggest that recognition memory aspects of the DNMS and DRST are largely dependent upon the integrity of the medial temporal lobe limbic system (including the entorhinal, perirhinal, and parahippocampal cortices) while other aspects such as rule learning (DNMS acquisition), working memory, and spatial location on the DRST are largely dependent upon the integrity of the lateral prefrontal cortex including area 46 of the sulcus principalis (Petrides, 2005).

Statistical methods

When comparing means between groups of parameters from our density map analysis tests with a significance level of $P=0.05$ two-tailed were used. Whenever testing for correlations between two sets of parameters the Spearman’s rank correlation coefficient was used. While a more traditional Pearson correlation calculation measures linear trends in data, the Spearman correlation coefficient was used to assess how well the relationship between two variables could be related to an arbitrary monotonic function without making any assumptions about the frequency distribution of the variables, i.e., without the assumption that the relationship between the variables is linear or requiring the variables to be measured on interval scales. For the correlation calculations a significance level to $P=0.05$ two-tailed was used.

RESULTS

As described in the Experimental Procedures section, we examined digital images acquired from ROIs from area 46 in the dorsal ($N=19$) and ventral banks ($N=16$) of sulcus principalis. We note that results from 14 of the 16 monkeys examined for ventral area 46 were studied previously, but
Table 1. Age, number of neurons, total area, and neuronal density for each of the female monkey brains analyzed

<table>
<thead>
<tr>
<th>Case</th>
<th>Age</th>
<th>Number of neurons</th>
<th>Area (microns²)</th>
<th>Density (neurons/μm²)</th>
<th>Number of neurons</th>
<th>Area (microns²)</th>
<th>Density (neurons/μm²)</th>
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<td>1168</td>
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<td>0.0014514</td>
<td>1076</td>
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<td>993</td>
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<td>860,583.14</td>
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Microcolumnar measures

Microcolumnar parameters. Similar to neuronal density, the measures of microcolumnar organization derived from the density maps, with the exception of microcolumnar strength (see below), do not show significant changes with age. In particular, Fig. 4b shows the microcolumnar width $W$. The population average is $W = 12.303 ± 0.552 \mu m$ with region averages $W_{d} = 12.592 ± 0.873 \mu m$ and $W_{v} = 11.959 ± 0.641 \mu m$ which are not significantly different. This figure also shows that there is no significant change of microcolumnar width with age.

The distance between microcolumns $P$ has a population average of $P = 26.183 ± 1.400 \mu m$. When comparing this by region we found that on average $P_{d} = 26.52 ± 1.92 \mu m$ and $P_{v} = 25.79 ± 2.11 \mu m$, which is not significantly different. Moreover, like $W$, there is no significant change in the distance between microcolumns with age as shown in Fig. 4c.

In Table 2, we show the individual neuronal density $\rho$ (in units of thousands of neurons per μm²) as a function of age. This graph shows that there is no significant effect of age on neuronal density confirming previous reports that neurons from area 46 are not lost with age (Peters et al., 1994; Smith et al., 2004).

Table 2. Average values of measures of microcolumnarity

<table>
<thead>
<tr>
<th></th>
<th>Dorsal</th>
<th></th>
<th>Ventral</th>
<th></th>
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</thead>
<tbody>
<tr>
<td>S</td>
<td>1.202</td>
<td></td>
<td>1.207</td>
<td></td>
</tr>
<tr>
<td>T</td>
<td>1.06</td>
<td>0.98</td>
<td>1.04</td>
<td>0.98</td>
</tr>
<tr>
<td>P</td>
<td>26.52 μm</td>
<td>25.79 μm</td>
<td>25.97 μm</td>
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</tr>
<tr>
<td>W</td>
<td>12.59 ± μm</td>
<td>11.95 ± μm</td>
<td>12.59 ± μm</td>
<td>11.95 ± μm</td>
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<tr>
<td>L</td>
<td>35.3 ± μm</td>
<td>40.5 ± μm</td>
<td>35.3 ± μm</td>
<td>40.5 ± μm</td>
</tr>
<tr>
<td>Y</td>
<td>18 ± μm</td>
<td>21 ± μm</td>
<td>18 ± μm</td>
<td>21 ± μm</td>
</tr>
<tr>
<td>ρ</td>
<td>0.001414 ± neu/μm²</td>
<td>0.001186 ± neu/μm²</td>
<td>0.001414 ± neu/μm²</td>
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*Significantly different, $P < 0.05$. 

only results on microcolumnar strength were reported (Cruz et al., 2004).
Fig. 4. Neuronal density and microcolumnar properties for all of the cases analyzed. Bullets correspond to dorsal area (N=19) while open squares correspond to the ventral area (N=16). The graphs correspond to (a) neuronal density $\rho$, (b) width $W$, (c) inter-column distance $P$, (d) degree of microcolumnar periodicity $T$, (e) length $L$, and (f) interneuronal distance of microcolumns $Y$. The lines are regression (best fit) lines. Asterisks indicate quantities that are significantly different between groups.

which are significantly different ($P=0.002$). However, when plotted as a function of age, $Y$ also does not show any significant age-related change (Fig. 4).

Microcolumnar strength. The average strength of microcolumns $S$ (ratio of the density of neurons in the microcolumn to the total density of neurons in the ROI) for the whole population is $<S>_D=1.204\pm0.013$. When grouping by region we obtain $<S>_D=1.202\pm0.020$ for 46D and $<S>_V=1.207\pm0.018$ for 46V with no statistically significant difference between the two areas, Fig. 5a and b shows $S$ per region vs. age and documents a significant age-related reduction in $S$ as a function of age in both area 46D and 46V (we plot $S-1$ instead of $S$ to obtain a quantity that is zero when there is no microcolumnarity). These data confirm our previous findings based on some of the same subjects for 46V (Cruz et al., 2004) and extend the observation of an age-related alteration in microcolumnar strength to the larger cohort in 46V as well as to 46D.

To test whether $S_D$ and $S_V$ correlate with each other we plot in Fig. 5c $S_V$ vs. $S_D$ where each point corresponds to one case and the axes correspond to the microcolumnar strength in the dorsal and ventral region of each case. Analysis of the scatterplot in Fig. 5c shows that there is no significant correlation between $S_D$ and $S_V$ within individuals, suggesting that despite the effect of age on the strength of microcolumns, this effect is not strongly correlated between the two areas within individual subjects.

Relationship to behavior

All of the behavioral tests considered in this paper are sensitive to age-related cognitive impairments (Herm, 1997). In our cohort of monkeys, however, only three behavioral tests show a significant change with age: (i) the CII (Fig. 6a), (ii) a spatial working memory span task, DRST spatial (Fig. 6b), and (iii) the acquisition at a 10 s delay of the DNMS test, DNMS-10 (Fig. 6c). The acquisition at a 2 min delay of the DNMS test, DNMS-120 (Fig. 6d) does not show changes with age in our cohort.

In Fig. 6e to l we show all of the cognitive tests versus $S_D$ and $S_V$. These plots show that there is no significant
DISCUSSION

Summary

In this paper we use a density map method to quantify the changing structure of microcolumns in two distinct parts of area 46—the dorsal and ventral banks of the middle level of sulcus principalis of the prefrontal cortex—in behaviorally characterized aging female monkeys. This quantitative analysis demonstrates that while many microcolumnar properties are similar between area 46 in the dorsal and ventral banks, there are differences in others. Moreover, while there are age-related effects in both areas, only the changes in ventral area 46 show a relationship with aging.

Basic properties of microcolumns

Our density map method allows us to quantify and explore changes in microcolumnar structure by assessing subtle alterations in the spatial organization of neurons. With this method we find that the microcolumnar strength S does not differ significantly between 46D and 46V. In contrast, we find significant differences between areas 46D and 46V in the global neuronal density \( n \), the effective microcolumn length \( L \), the degree of microcolumnar periodicity \( T \), and the interneuronal vertical distance \( Y \) within the microcolumns. Specifically, 46D has an overall greater neuronal density and exhibits a stronger degree of microcolumnar periodicity than 46V. In contrast, 46V exhibits longer effective microcolumnar lengths \( L \) and increased distance between neurons within microcolumns \( Y \). A possible explanation for the coincident larger \( L \) and \( Y \) values in 46V is that both of these measures depend on the angle of cut of the tissue sections, i.e., perpendicular cuts to the pla may preserve longer columns and record larger \( Y \) distances due to the smaller projection angle as opposed to more oblique cuts. Because of intrinsic experimental difficulties in determining the angle of cut of each section, we are currently investigating the correlation between \( L \) and \( Y \) as a function of angle of cut by means of our three-dimensional computational model (Cruz et al., 2008).

Microcolumnar properties and age

While the microcolumnar strength does not differ between 46D and 46V, there is a significant age-related reduction in \( S \) for both areas. This reduction in microcolumnar strength is more than a statistical property of the tissue and can be visualized as a literal loss of microcolumnar organization. In Fig. 7 we show two images from each of three cases studied here for which their values of \( S \) span the numerical range observed in this study. The top row consists of two images depicting a case with stronger microcolumnarity \((a-b)\) than the other two cases \((c-d) \) and \((e-f)\), indicated by the numerical value of \( S \) for each case.

Interestingly, even though \( S \) declines with age in both areas, only in 46V does this decline correlate with the CII, a composite measure of the impairment indicated by the DNMS-10, DNMS-120, and DRST spatial scores. By comparing \( S_y \) with \( S_d \) we further show that these two quantities do not correlate with each other, suggesting that although.
Fig. 6. Plots of cognitive tests vs. age, $S_D$, and $S_V$. The tests include: a global CII, a spatial working memory span task (DRST-spatial), the acquisition at a 10 s delay of the DNMS-10, and recognition memory performance of DNMS-120. Plots showing significant correlations are marked with thick linear fits. For the monkeys from our cohort, only (a) CII, (b) DRST-spatial, and (c) DNMS-10 correlate with age. Correlations between behavior and $S$ show that CII correlates with $S_D$, (d).

Both decay with age, the two changes are independent and only $S_V$ correlates with the cognitive tests considered in this paper. The fact that the age-related loss of microcolumnar strength in 46V does not correlate with the age-related behavioral impairments in this group of subjects while the loss of strength in 46V does, likely reflects the differential function of these two areas. Petrides (2000b, 2005) reviewed data on both the differential function and connectivity of 46D and 46V and concluded that these two areas had specialized functions. While the exact nature of these functions still remains to be determined, the executive function and temporal ordering that Petrides ascribed to 46D seems unlikely to be directly tapped by the battery of memory tasks available on this cohort of subjects. In contrast, the involvement in memory functions that Petrides ascribed to 46V is supported by the behavioral deficits observed in our cohort of subjects.

Insights into possible mechanisms using a computational approach

The reported age-related change in microcolumnar strength $S$ raises the question of possible mechanisms underlying this change. There are numerous neurobiological variables that could be related to this change. To gain some insight into which of these variables are relevant, and thus possible underlying mechanism(s), we previously developed a two-dimensional computational model (Cruz et al., 2004) to explore possible relationships between loss of microcolumnar strength and age-related changes in neuronal locations in the cortex given that the number of neurons and neuronal density do not change with age. Using this model, we demonstrated that the observed reduction of microcolumnar strength in 46V observed in aged monkeys could be accounted for if neuron locations in layer 3 of an average young monkey were computationally displaced by small random increments with an
Potential biological mechanisms

Based on our findings, it is plausible that the observed changes in microcolumnar measures reflect subtle age-related displacements in neuron location. Although this conclusion by itself does not establish a biological mechanism for such displacements, it suggests a number of possible candidates. It is clear that the brain is dynamic and changing throughout the lifespan. Synapses change morphology on a time scale of minutes to hours in response to long-term potentiation (Leuner et al., 2003; Lynch, 2004; Lambrecht and LeDoux, 2004). Synaptic plasticity is also seen in response to hormonal manipulations, though the time scale for morphological changes is on the order of a month (Weeks, 2003). Dendrites also sprout or regress (Weeks, 2003; Grill and Riddle, 2002). There is growing evidence that even in the adult brain new neurons are generated and integrate themselves into functional circuits (Shors et al., 2001). Furthermore, while it is increasingly difficult to find evidence for significant neuronal loss in normal aging, the literature on aging provides evidence of a variety of changes in layer 3 neurons in the aging brain. Page et al. (2002), Duan et al. (2003), Morrison and Hof (2007) and Dickstein et al. (2007) have all documented dendritic atrophy including changes in branching patterns and spine density. In addition, while basic membrane properties of layer 3 pyramidal neurons are preserved, there are changes in synaptic input and action potential generation that also indicate significant alterations in function (e.g., Luebke et al., 2004; Chang et al., 2005). There are also a variety of degenerative changes in myelin (Peters 2002), and even loss of corticocortical axons (Sandell and Peters, 2003), which generally originate from layer 3 where our measurements were conducted. Consequently, it is plausible that one or more of these brain changes contribute to the observed subtle displacements of neurons and may underlie our measured changes in the strength of microcolumnar connectivity.

CONCLUSION

The quantitative density map approach utilized in this study demonstrates age-related changes in measures of microcolumnar innervation in area 46 of the prefrontal cortex. Our study shows that there is an age-related loss of microcolumnar strength in both 46D and 46V, however, only the loss of microcolumnar strength in 46V is associated with age-related cognitive impairment in a global CII that assesses learning, recognition memory, and working memory. These data are also consistent with the view that a loss in the strength of microcolumns is a consequence of subtle changes in the organization of neurons within microcolumns, but because subtle changes in complex systems can cause significant changes due to their interconnectivity and cooperativity, these changes may have functional consequences for information processing in the aging brain. The data also demonstrate that even within parts of the same cytoarchitectonic area, there may be further subtle anatomical and functional dissociations that are discernible with the methods we have developed. Finally, if the reductions in S with age in both areas exami-
ined reflect a more global age-related disruption that results in a loss of neuronal coherence within microcolumns throughout the brain, the lack of correlation with age-related cognitive impairment for the measures of 4D implies that our battery of behavioral tests is insensitive to the functional changes in that area.

Software

The code containing the density map method used in this work to quantify microcolumns is available in the download section of www.physics.drexel.edu/~cocruz/micros/.

Acknowledgments—This work was supported by the National Institutes of Health grants R01-AG021133, P01-AG000001 and P51-RR01165, the Alzheimer Association, and the Bechtel Foundation.

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(Revised 18 November 2008)
(Accepted 27 November 2008)