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NEW TECHNIQUES FOR IMAGING, DIGITIZATION AND ANALYSIS OF THREE-DIMENSIONAL NEURAL MORPHOLOGY ON MULTIPLE SCALES

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Abstract—Cognitive impairment in normal aging and neurodegenerative diseases is accompanied by altered morphologies on multiple scales. Understanding of the role of these structural changes in producing functional deficits in brain aging and neuropsychiatric disorders requires accurate three-dimensional representations of neuronal morphology, and realistic biophysical modeling that can directly relate structural changes to altered neuronal firing patterns. To date however, tools capable of resolving, digitizing and analyzing neuronal morphology on both local and global scales, and with sufficient throughput and automation, have been lacking. The precision of existing image analysis-based morphometric tools is restricted at the finest scales, where resolution of fine dendritic features and spine geometry is limited by the skeletonization methods used, and by quantization errors arising from insufficient imaging resolution. We are developing techniques for imaging, reconstruction and analysis of neuronal morphology that capture both local and global structural variation. To minimize quantization error and evaluate more precisely the fine geometry of dendrites and spines, we introduce a new shape analysis technique, the Rayburst sampling algorithm that uses the original grayscale data rather than the segmented images for precise, continuous radius estimation, and multidirectional radius sampling to represent non-circular branch cross-sections and anisotropic structures such as dendritic spine heads, with greater accuracy. We apply the Rayburst technique to 3D neuronal shape analysis at different scales. We reconstruct and digitize entire neurons from stacks of laser-scanning microscopy images, as well as globally complex structures such as multineuron networks and microvascular networks. We also introduce imaging techniques necessary to recover detailed information on three-dimensional mass distribution and surface roughness of amyloid beta plaques from human Alzheimer's disease patients and from the Tg2576 mouse that expresses the "Swedish" mutation of the amyloid precursor protein.

By providing true three-dimensional morphometry of complex histologic structures on multiple scales, the tools described in this report will enable multiscale biophysical modeling studies capable of testing potential mechanisms by which altered dendritic structure, spine geometry and network branching patterns that occur in normal aging and in many brain disorders, determine deficits of functions such as working memory and cognition.

Key words: confocal laser scanning microscopy, computational neurobiology, image analysis, dendritic spines, neurodegenerative disorders, microvasculature.

We are developing high-resolution three-dimensional (3D) techniques for analyzing the structural basis of impaired neural transmission and plasticity that underlies cognitive decline in normal aging and neurodegenerative disease. Such pathologic states are characterized by a progressive decline in memory-related cognitive abilities (Albert, 1996; Du et al., 2001; Chetelat et al., 2002; Small et al., 2002) and are accompanied by morphologic changes on multiple scales, from the fine-grained geometry of individual neurons, dendrites and spines (Anderson and Rutledge, 1996; de Brabander et al., 1998; Duan et al., 2000, 2003; Page et al., 2002; Jacobs et al., 2001; Hao et al., 2004; Hof and Morrison, 2004; Radley et al., 2005) to the spatial complexity of multineuron and vascular networks in the presence of space-occupying histopathologic lesions (Le et al., 2001; Stern et al., 2004). Inferring mechanisms by which these structural changes bring about the observed cognitive deficits requires a combination of precise 3D morphometry and biophysical modeling that can reliably represent both global dendritic structure and detailed spine geometry. Theoretical and technological developments over the past decades now allow for realistic modeling of structure–function relationships on multiple scales. The application of cable theory (Rall, 1962, 1964) to multicompartment modeling (Holmes and Rall, 1992; Hines,

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Abbreviations: AD, Alzheimer's disease; CLSM, confocal laser-scanning microscopy/microscope; DAPI, 4,6-diamidino-2-phenylindole; DLA, diffusion-limited aggregation; eGFP, enhanced green fluorescent protein; MLBD, median lower band diameter; MPLSM, multiphoton laser-scanning microscopy; n.a., numerical aperture; VIAS, Volume Integration and Alignment System; WD, working distance; 2D, two-dimensional; 3D, three-dimensional.

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1994; Bower and Beeman, 1998; Koch, 1999) for example, permits simulation of voltage spread throughout a branching dendritic tree of arbitrary complexity. High-resolution laser-scanning microscopy can resolve structures on scales ranging from entire neurons to dendritic spines spanning less than a micrometer. With the increasing speed of standard laboratory computers, digitizing, reconstructing and simulating these structures no longer requires supercomputing resources, and will become an increasingly important tool in understanding the structural determinants of normal and pathologic neuronal function.

At the single cell level, dendritic morphology is a fundamental determinant of synaptic integration and neural firing patterns (Mainen and Sejnowski, 1996; Stuart et al., 1999; Koch and Segev, 2000; Euler and Denk, 2001; Vetter et al., 2001; Henry et al., 2002; Krichmar et al., 2002; Ascoli, 2003; Häusser and Mel, 2003). Dendritic arborization patterns provide a substrate both for spatiotemporal integration of multiple inputs and the connectivity patterns that shape network dynamics. Compartmental modeling studies have demonstrated that model neurons with common ion distributions, differing only in the extent of arborization of their dendritic geometries, can reproduce the full range of firing patterns observed experimentally (Mainen and Sejnowski, 1996). Importantly, since both macroscopic branching topology and microscopic surface irregularities including dendritic varicosities (Surkis et al., 1998) and spine density, shape and distribution (Wilson, 1988; Holmes, 1989; Stratford et al., 1989; Baer and Rinzel, 1991; Tsay and Yuste, 2001; Yuste and Bonhoeffer, 2001; Nimchinsky et al., 2002) contribute to the normal case, accurate reconstruction and analysis of 3D structural alterations in neurodegenerative disease are essential to understanding the etiology of impaired cognitive function that accompanies such pathology.

While the gross morphologic effects of neurodegenerative disease have been well documented by stereologic approaches, alterations in fine-grained single neuron morphology and the global topology of multineuron and vascular networks remain poorly characterized. Accumulating data indicate that these more complex morphologic changes, and their interactions, are essential to understanding the spatiotemporal progress of neurodegenerative diseases (Le et al., 2001; Urbanc et al., 2002; D'Amore et al., 2003; Tsai et al., 2004). In Alzheimer's disease (AD) for example, amyloid beta protein exists in many molecular forms, and eventually aggregates into the insoluble plaques that are one of the neuropathological hallmarks of AD. Insoluble amyloid deposits comprising extracellular accumulations of amyloid beta peptide and other proteins surrounded by degenerating axons and dystrophic dendrites are an example of general histopathologic lesions that can physically distort neuronal processes in their vicinity, which must take tortuous routes around the lesions, resulting in characteristic distortions of local and global dendritic geometry (Knowles et al., 1999; Le et al., 2001; Urbanc et al., 2002). In addition, spines are often lost as spiny dendrites traverse a plaque, dendritic shafts undergo atrophy and axons in the vicinity of plaques develop varicosities (Tsai et al., 2004). These distortions and nonhomogeneous degenerative processes have not been quantified in 3D, and their effects on neuronal function are not known.

Severe vascular modifications are also detected in several neurodegenerative disorders (Buée et al., 1994; Bailey et al., 2004). Electron microscopy studies have revealed thickening and vacuolization of the vascular basement membrane, thinning of microvessels referred to as atrophic or string vessels (Bell and Ball, 1981), increased tortuosity (Fischer et al., 1990), and fragmentation of the microvasculature related to a decrease in the number of long microvessels and their branches (Buée et al., 1994). Many microvascular abnormalities occur prior to the development of clinical dementia and to the formation of the characteristic neuronal AD-type lesions (Buée et al., 1994; Keuker et al., 2000), emphasizing the importance of characterizing the spatiotemporal interactions of these alterations.

Current techniques for digitizing neuronal morphology in 3D entail manual tracing using custom packages such as NeuroZoom (Bloom et al., 1997) or Neurolucida (MicroBrightField, Williston, VT, USA). Such methods introduce systematic inaccuracies depending upon the individual performing the tracing, and fail to capture fine dendritic structure including varicosities, continuous taper and spine morphology. In particular, spines are represented as points, labeled independently of the dendritic tracing with a marker of uniform size. Relative diameters of spine heads and necks cannot currently be resolved with these software applications. While a few techniques of varying degrees of automation have been suggested for digitizing neuronal morphology from laser-scanning microscopy images (Streekstra et al., 1999, 2000; Messerli, 2000; He et al., 2003; Schmitt et al., 2004; and see van Pelt et al., 2001 for review), the limited size of the data sets that can be handled, and the general requirement for extensive manual editing renders these impractical in most cases for reconstructing entire neurons or multicellular structures at high resolution. New developments in image analysis and visualization are needed to provide faster, more accurate adaptive segmentation and automatic reconstruction with minimal user interaction, for use with large data sets. A particular problem with existing image analysis-based morphometric tools is their restricted precision at the finest scales, where resolution of fine dendritic features and spine geometry is limited by the skeletonization methods used, and by quantization errors. Even when imaged at the limits of light microscopy, single spines may span as few as three to 10 pixels, and neck diameters may be subvoxel resolution. The alternative to light microscopic approaches, electron microscopy, provides the greatest accuracy at these fine scales (Harris and Stevens, 1988; Harris et al., 1992; Harris and Kater, 1994) but would be prohibitively time consuming for reconstruction of entire dendritic trees or multicellular structures. Accurate recovery of fine neuronal geometry thus requires new skeletonization and analysis algorithms capable of subvoxel resolution at the light microscopic level. A primary goal of our