

a substantial percentage of patients, especially if no predisposing factors were present.^{3,7,8} However, the period of follow-up in these early studies was rarely more than 1 year. The presence of thalamic hemorrhage has been associated with a particularly poor outcome, with 10 of 12 demonstrating findings of cerebral palsy at 18 months.² The presence, size, and location of venous infarction should also be an important factor in predicting outcome. Studies focusing on the severity of parenchymal brain injury, rather than on IVH, with longer periods of follow-up are needed to determine predictors of neurological sequelae after IVH among term newborns.

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Quantitative Analysis of Cortical Pyramidal Neurons after Corpus Callosotomy

Bob Jacobs, PhD,¹ Johanna Creswell, BA,¹ Jonathan P. Britt, BA,¹ Kevin L. Ford, PhD,¹ Joseph E. Bogen, MD,^{2,3} and Eran Zaidel, PhD⁴

This study quantitatively explored the dendritic/spine extent of supragranular pyramidal neurons across several cortical areas in two adult male subjects who had undergone a callosotomy several decades before death. In all cortical areas, there were numerous atypical, supragranular pyramidal neurons with elongated “tap root” basilar dendrites. These atypical cells could be associated with an underlying epileptic condition and/or could represent a compensatory mechanism in response to deafferentation after callosotomy.

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From the ¹Laboratory of Quantitative Neuromorphology, Department of Psychology, Colorado College, Colorado Springs, CO; ²Neurological Surgery, University of Southern California, Los Angeles; ³Department of Biology, California Institute of Technology, Pasadena; and ⁴Department of Psychology, University of California, Los Angeles, CA.

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Address correspondence to Dr Jacobs, Laboratory of Quantitative Neuromorphology, Department of Psychology, Colorado College, 14 East Cache La Poudre, Colorado Springs, CO 80903. E-mail: bjacobs@coloradocollege.edu

This study quantitatively examines the dendritic systems of cortical areas several decades after a corpus callosotomy. As such, it supplements previous research on the relationship between dendritic extent and cortical function¹ and, more importantly, constitutes an unprecedented opportunity to explore in humans the potential long-term cortical correlates of a callosotomy. Previous research in neurologically normal individuals has shown a stepwise increase in dendritic complexity as the integrative demands on cortical regions increase; for example, motor cortex tends to be less dendritically complex than Broca's area, which, in turn, is less complex than prefrontal regions.² In terms of callosotomy effects on cortical pyramidal neurons, some morphometric studies on nonhuman animals have documented no acute changes to these callosal neurons,^{3,4} whereas others have noted acute degenerative changes (eg, loss of dendritic spines).⁵ No studies to our knowledge have investigated long-term effects vis-à-vis pyramidal neurons in layer III of human cerebral cortex.

These layer III projection neurons constitute the major origin and target of callosal fibers,⁶ thus playing a critical role in interhemispheric integration. This study quantifies their basilar dendritic systems in three cortical regions across both hemispheres: primary motor cortex (Brodmann's area [BA] 4), Broca's area (BA44), and dorsolateral prefrontal cortex (BA10). In addition to documenting potential regional and hemispheric differences, this study explores the possibility that supragranular cortical neurons may undergo long-term morphological changes with section of the corpus callosum.

Subjects and Methods

Subjects

Tissue was obtained from two male subjects (L.B. and R.Y.), whose cases have been extensively documented.^{7,8} In both subjects, cerebral callosotomy included division of the entire corpus callosum and the anterior commissure; the massa intermedia was not visualized during surgery. L.B. exhibited normal development until his first convulsion at age 3.5 years; his condition subsequently worsened until he was having generalized convulsions approximately once a week during the year before his surgery. Repeated electroencephalograms indicated borderline slowing, which was particularly evident bitemporally. Cerebral callosotomy was performed when he was 13 years old. The postoperative period was largely uneventful, and he remained generally convulsion-free until his death at age 47 years from a cerebellar hemorrhage.

R.Y. began having generalized convulsions at age 17 years. Brief auras of visual alteration suggested a posterior focus, but electroencephalograms were nonlocalizing. Cerebral callosotomy was performed when he was 43 years old. His postoperative course was largely unremarkable, and he was largely seizure-free in the last 20 years of his life, dying at age 72 years with squamous carcinoma that had metastasized into his brain. Before staining, L.B.'s brain had been formalin

fixed for 18 months; R.Y.'s brain had been fixed for 6 years. Tissue was provided by Drs Harry Vinters and A. B. Scheibel of the UCLA Medical Center. The research protocol was approved by the Colorado College Human Subjects Review Board (H94-004).

Tissue Processing and Quantification

Representative tissue blocks were removed at autopsy from three cortical areas of both hemispheres: BA4, BA44, and BA10. Before quantification, coded tissue blocks were processed with the Braitenberg variation of the Golgi-Kopsch method,⁹ which differs from the rapid Golgi method we typically use.^{2,10} Although the rapid Golgi variation provides somewhat better impregnation than the Golgi-Kopsch method, it does not work as well as the Golgi-Kopsch method for tissue, such as in our samples, with long fixation periods.¹¹ Processed tissue was serially sectioned at 120 μ m with a vibratome. Fifteen relatively isolated supragranular pyramidal cells per tissue block were randomly chosen for analysis following previously established criteria.^{2,10} In the course of quantifying these pyramidal cells on a NeuroLucida system (MicroBrightfield, Williston, VT), it became apparent that the tissue from both brains also contained several atypical pyramidal neurons, primarily in deep layer III. Consequently, 34 of these atypical supragranular neurons meeting selection criteria^{2,10} were subsequently quantified. Unfortunately, these cells could be quantified only for L.B. because of poor impregnation in R.Y. Dependent measures were (1) total dendritic length (summed length of dendritic segments), (2) mean segment length (average length of segments), (3) dendritic segment count (number of segments), (4) dendritic spine number (sum of all spines on segments), and (5) dendritic spine density (average number of spines per micrometer of dendritic length). Details of this methodology have been well documented elsewhere.^{2,10}

Results

Typical Pyramidal Neurons

In general, dendritic systems in the left hemisphere tended to increase in complexity from BA4, to BA44, and BA10. In the right hemisphere, BA44 was generally the most complex region, followed by BA10 and BA4. There was no significant overall difference between hemispheres. In a nested analysis of variance design (SAS, V8 for Windows; SAS Institute, Cary, NC), there was a significant difference across the three Brodmann's areas for total dendritic length [$F(4,84) = 5.4$, $p < 0.0006$], mean segment length [$F(4,84) = 18.04$, $p < 0.0001$], dendritic segment count [$F(4,84) = 18.1$, $p < 0.0001$], and dendritic spine number [$F(4,84) = 7.59$, $p < 0.0001$], but not for dendritic spine density.

Atypical Pyramidal Neurons

Atypical, supragranular pyramidal neurons were present near the layer III/IV junction across all areas (left BA10, four cells; right BA4, two cells; left BA10, five cells) and particularly abundant in left BA44 ($n = 23$;

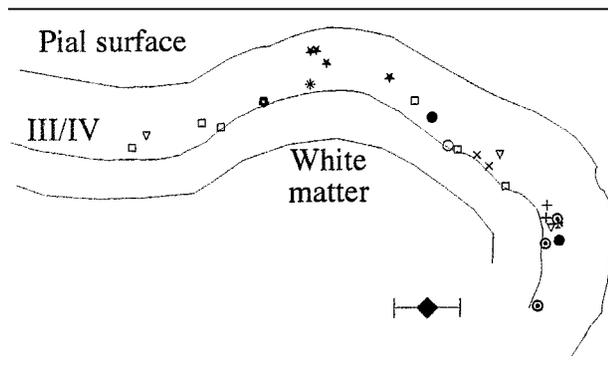


Fig 1. Cortical cross-section of Brodmann's area (BA) 44 of the left hemisphere in L.B. illustrating the relative position of the 23 atypical neurons in this cortical region. These cells were evenly distributed in deep layer III. Each symbol represents a different neuron, with the different symbols corresponding to different 120 μ m sections overlaid to comprise Broca's area. Scale bar = 2,000 μ m.

Fig 1). Quantitatively, these neurons were approximately as complex in dendritic length and spines as the typical pyramidal cells. The main characteristic of these cells was the presence of a "tap root" basilar dendrite, that is, a single, elongated branch that descended obliquely into deeper cortical laminae. These tap roots, characterized by numerous secondary branches, were

significantly more complex than the other basilar dendrites on all measures [total dendritic length: $t(35) = 14.3$, $p < 0.001$; dendritic segment count: $t(35) = 13.6$, $p < 0.001$; dendritic spine number: $t(35) = 11.1$, $p < 0.001$; dendritic spine density: $t(35) = 8.4$, $p < 0.001$] except mean segment length (Figs 2 and 3). On average, the tap root dendrite constituted at least 60% of each cell's overall dendritic length and approximately 72% of the cell's basilar dendritic spine number.

Discussion

Despite the typical methodological limitations that characterize such quantitative investigations (eg, the practical constraints of human research, Golgi stains, small sample sizes, and problems of prolonged fixation times),^{1,2,10} our results for typical pyramidal neurons are consistent with previous dendritic findings. As in our results, quantitative hemispheric differences in dendritic systems appear to be minimal.¹ The obtained regional differences in the left hemisphere (BA4 < BA44 < BA10) reflect documented cortical hierarchies in dendritic complexity,² although the absolute dendritic measures are attenuated because of the prolonged fixation time and to differences between the rapid Golgi and the Golgi-Kopsch stains. The obtained regional

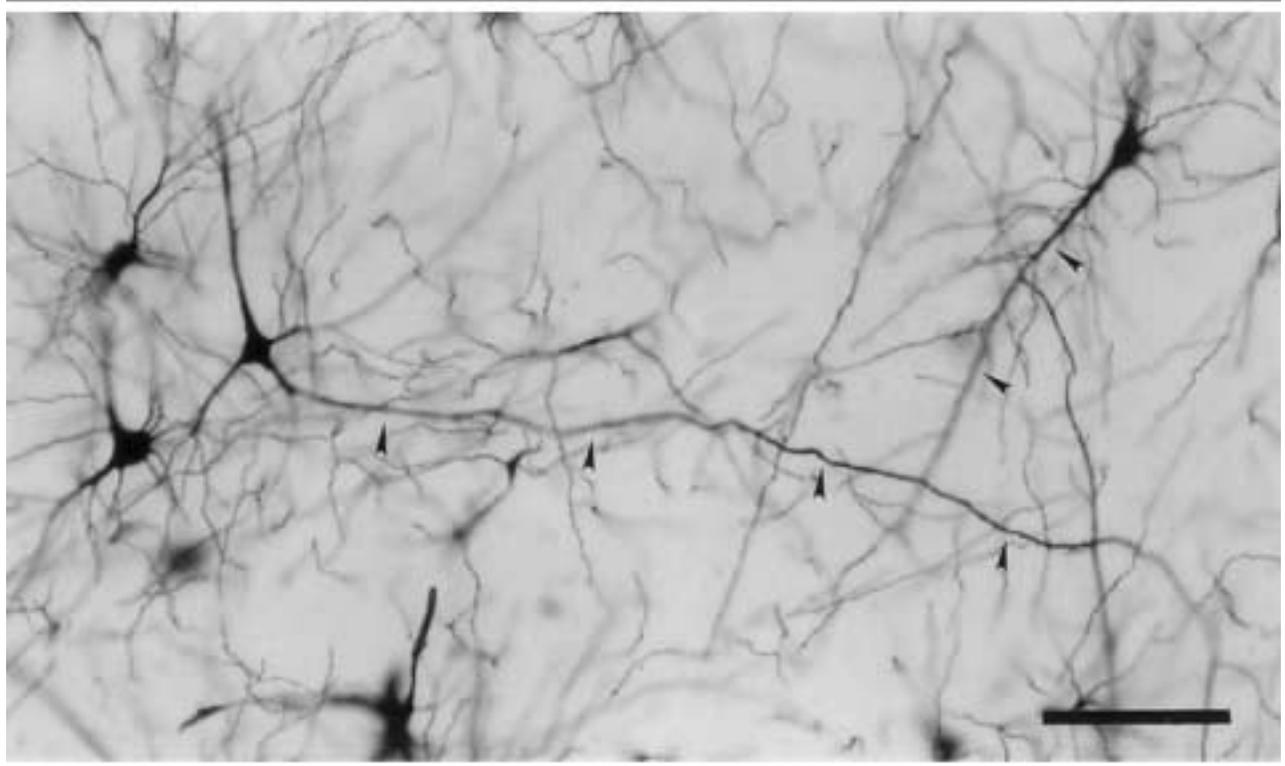


Fig 2. Photomicrograph of atypical supragranular pyramidal cells from Broca's area in the left hemisphere of case L.B., with the pia mater at the top. Note the two extensive, asymmetrical "tap roots" (arrowheads) extending ventrolaterally from the base of the somata. Scale bar = 100 μ m.

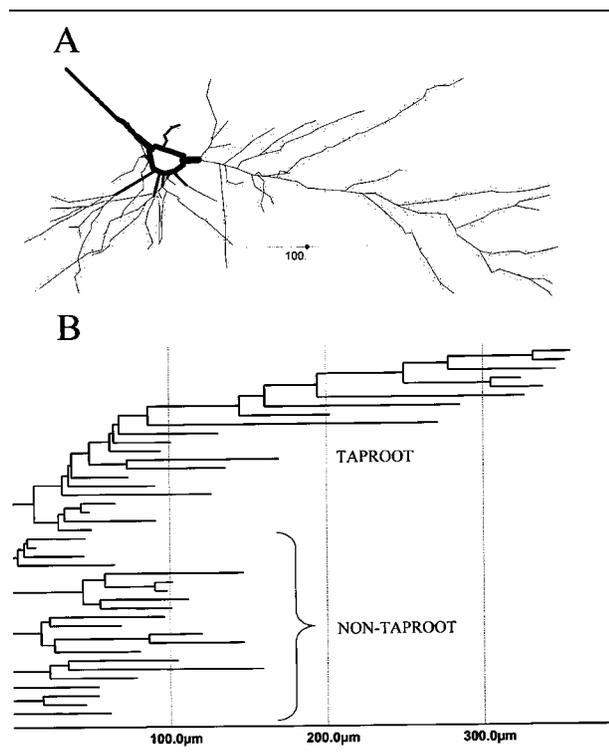


Fig 3. A representative tracing (A) of an atypical pyramidal neuron from Broca's area of the left hemisphere in case L.B. with its complementary dendrogram (B). The apical dendrite of the tracing extends upward, with the tap root extending ventrolaterally from the right, basal pole of the soma. The dendrogram indicates the substantially more complex nature of the "tap root" dendrite as compared with the corresponding basilar dendrites.

differences in the right hemisphere, with BA44 being most complex, are more difficult to explain, but may be a consequence of the small sample size.

The spine loss and nodular dendritic swellings that often characterize epileptic tissue¹² were not apparent in our samples. However, abundantly present were atypical, supragranular pyramidal neurons, with elongated basilar tap roots reminiscent of those observed in Betz cells.¹³ Indeed, in quantifying more than 100 neurologically normal cases, we have never encountered such atypical cells. Although these were present in all areas examined, they were clearly most abundant in left hemisphere Broca's area for reasons that remain unclear. Such atypical neurons have been qualitatively documented, however, in biopsied cortical tissue from epileptic patients.¹⁴ Belichenko and colleagues refer to these as "dinosaur-like" cells, and suggest that they are a possible result of microdysgenesis. Although present in normal cortical areas, such abnormal pyramidal cells predominated in epileptogenic zones, but only in infragranular layers.¹⁴ These atypical cells could, in conjunction with losses of inhibitory interneurons,¹⁵ significantly contribute to the electrical abnormalities in

epileptogenic tissue. Thus, one cannot exclude the possibility in this study that the atypical, supragranular cells were present before the callosotomy and indeed contributed to the epileptic condition that made surgical treatment necessary.

Another, not mutually exclusive, possibility that deserves consideration is that these elongated tap roots are, in fact, a reactive consequence of the callosotomy itself. Several lines of evidence support this alternative. First, to the extent that the epileptogenic focus was localized to the temporal lobe, our tissue samples did not appear to be from epileptogenic tissue. Second, a disproportional number of callosal axons terminate at the layer III/IV junction, preferentially on the dendritic systems of deep, supragranular pyramidal neurons.¹⁶ As such, the pyramidal neurons in this study may have suffered substantial deafferentation after callosotomy. Third, supragranular pyramidal cells are sensitive integrators of corticocortical activity,¹⁷ with dendritic/spine systems that appear particularly malleable in response to internal and external environmental changes.¹⁸ Finally, consistent with deafferentation research indicating that damage to afferent structures can trigger reactive synaptogenesis and dendritic restructuring,¹⁹ the observed basilar tap roots exhibited significantly higher spine density over their accompanying basilar dendrites, constituting the major source of synaptic input for the cell. Thus, the extensive tap root may serve as a compensatory mechanism for the loss in connectivity after callosotomy. In conclusion, only further long-term research on the morphological consequences of callosotomy can begin to address these issues. A particularly appealing population would be those subjects with partial callosotomies (ie, sparing the splenium),²⁰ which would allow dendritic comparison of deafferented anterior with intact posterior cortical regions.

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Ciliary Neurotrophic Factor Genotype Does Not Influence Clinical Phenotype in Amyotrophic Lateral Sclerosis

Ammar Al-Chalabi, PhD, MRCP,^{1,2}
 Margaret D. Scheffler, BA,² Bradley N. Smith, BSc,¹
 Matthew J. Parton, PhD, MRCP,¹
 Merit E. Cudkowicz, MD,³ Peter M. Andersen, DMSc,⁴
 Douglas L. Hayden, MA,⁵ Valerie K. Hansen, BSc,¹
 Martin R. Turner, MRCP,¹
 Christopher E. Shaw, MD, FRCP,⁶
 P. Nigel Leigh, PhD, FRCP,¹
 and Robert H. Brown, Jr, MD, DPhil²

Ciliary neurotrophic factor (CNTF) maintains survival of adult motor neurons. Mice lacking the *CNTF* gene develop mild, progressive motor neuron loss. In the normal human population, 1 to 2.3% are homozygous for a null allele, and reports suggest this mutant is associated with a younger onset of amyotrophic lateral sclerosis (ALS). We have tested this hypothesis in a study of 400 subjects with ALS and 236 controls. There was no difference in age of onset, clinical presentation, rate of progression, or disease duration for those with one or two copies of the null allele, excluding CNTF as a major disease modifier in ALS.

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Amyotrophic lateral sclerosis (ALS) is a disease in which there is progressive degeneration of motor neurons resulting in paralysis and death, usually over approximately 3 years. Approximately 10% of cases show autosomal dominant inheritance. Mutations in the gene for Cu/Zn superoxide dismutase (SOD1) account

From the ¹Department of Neurology, Academic Neuroscience Centre, Institute of Psychiatry, King's College London, London, United Kingdom; ²Day Neuromuscular Research Laboratory, MGH East, Charlestown, MA; ³Neurology Clinical Trials Unit, Massachusetts General Hospital, Boston, MA; ⁴Department of Neurology, Umeå University, Umeå, Sweden; ⁵General Clinical Research Center, Massachusetts General Hospital, Boston, MA; and ⁶Departments of Neurology and Medical and Molecular Genetics, Guy's King's and St. Thomas' School of Medicine, and Institute of Psychiatry, King's College London, United Kingdom.

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Address correspondence to Dr Al-Chalabi, Department of Neurology, PO41, Institute of Psychiatry, De Crespigny Park, London SE5 8AF, UK. E-mail: ammar@iop.kcl.ac.uk