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SHORT ORIGINAL COMMUNICATION

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Hippocampus in autism: a Golgi analysis

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Abstract Autism is a behaviorally defined syndrome in which neuropathological abnormalities have been identified in the limbic system and cerebellum. The morphology of hippocampal neurons in two cases of infantile autism was studied and compared to age-matched controls. CA4 neurons in autistic children were smaller in perikaryon area and dendritic branching of both CA4 and CA1 neurons was less than in controls. These findings are consistent with previous studies and suggest a curtailment in maturation in the pathogenesis of autism.

Key words Autism · Hippocampus

Introduction

Autism is a behaviorally defined syndrome characterized by atypical social interaction, disordered verbal and nonverbal communication, restricted areas of interest, limited imaginative play, and a need for sameness. Neuroanatomical abnormalities have been observed in the limbic system and cerebellum. In the former, increased neuronal cell packing density and decreased nerve cell size have been found bilaterally in the amygdala, entorhinal cortex, mammillary body, anterior cingulate gyrus, the medial septal nucleus, and throughout the hippocampal complex [2]. In the cerebellum, decreased numbers of Purkinje cells and abnormalities in the cerebellar nuclei have been observed [3]. Despite these consistent findings, no investigations of the dendritic development of individual neurons have been reported. The purpose of this study is to describe the dendritic morphology of hippocampal neurons, as seen with the Golgi stain, in two cases of well-documented autism in comparison with controls.

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Case reports

Case 1

Case 1 was a male child born after a normal pregnancy, labor, and delivery. Although motor milestones were normal, failure to develop language was noted at 2 years of age. By 30 months of age, he had a few single words, echolalia, a flat affect, and inappropriate laughter. He made no eye or social contact with other people, engaged in repetitive self-stimulatory behavior and perseverative play, and was described as hyperactive. He never experienced seizures nor did he receive any medication. At the age of 9 years, he was found dead in bed. No cause of death was found at autopsy.

Case 2

This girl was born following an uncomplicated pregnancy, labor, and delivery. She walked independently at 10 months of age, spoke single words at 1 year, and could recite the alphabet by 18 months. At this point, however, her language acquisition ceased and her behavior became unusual. She became socially withdrawn, engaged in perseverative play, and her speech consisted of single words, jargon, and echolalia. She never experienced a seizure and received no medication. Computerized tomographic scans of the head, electroencephalograms, extensive metabolic studies, and chromosomal analysis for Fragile X were negative. Similar studies were negative in her older brother who had been diagnosed with mental retardation and autism. At the age of 7 years, death occurred by accidental drowning.

Materials and methods

The brains were fixed in a buffered formalin solution. Blocks were taken from the hippocampus and stained using the rapid Golgi method. The blocks measuring 1 cm^3 were placed in a solution of 1 g osmium tetroxide, 8 g potassium dichromate, and 300 ml distilled water for 5 days. After fixation, the blocks were rinsed in 0.75% silver nitrate and silvered for 24 h in a fresh 0.75% silver nitrate solution. The blocks were dehydrated, embedded in low-vis-

cosity nitrocellulose, and cut at a thickness of 120 mm with a sliding knife microtome. Sections were cleared through ethanol, terpinol, and xylene and mounted under glass cover slips with Permount.

Golgi-stained sections from two controls, ages 8 and 13 years, were chosen from a collection of similarly stained material from the Southard Laboratory of the Fernald State School, Waltham, Massachusetts. The controls were selected on the basis of proximity in age and the availability of well-stained, comparable hippocampal neurons.

Neurons in the hippocampal CA1 and CA4 fields of the autistic and control material were identified and drawn with the aid of a camera lucida at a magnification of × 1000. Using a digital planimeter, the perikaryon area of each nerve cell was measured. The complexity of dendritic arborization was determined in the camera lucida drawings using the concentric circles method of Sholl [15], the circles being spaced 50 mm apart. Two-tailed unpaired *t*-tests were performed on the perikaryon areas and branching of the CA1 and CA4 pyramidal neurons.

Results

Due to unavoidable variability in staining with the Golgi method, case 1 demonstrated good CA4 neuronal staining, whereas case 2 did not. Both cases had adequate CA1 pyramidal neuron staining (Fig. 1). Perikaryon area and branching were determined only on intact neurons. Six CA1 neurons from the autistic cases were compared to five CA1 neurons from age-matched controls and five CA4 neurons from the autistic case 1 were compared to six similar neurons from a control.

The cell bodies of the CA4 neurons of the autistic patient were significantly smaller ($P < 0.01$) in area (mean 551.0 ± 41.1 mm^2) than those of the 8-year-old age-matched control (mean 757.1 ± 113.9 mm^2). The perikaryon area of the CA1 neurons of both autistic children was not significantly different from the controls (Table 1). The CA4 and CA1 pyramidal neurons of the autistic patients had significantly less branching compared with controls as determined by the number of dendrites crossing each concentric circle (Figs. 2, 3). No dysmorphic features were identified.

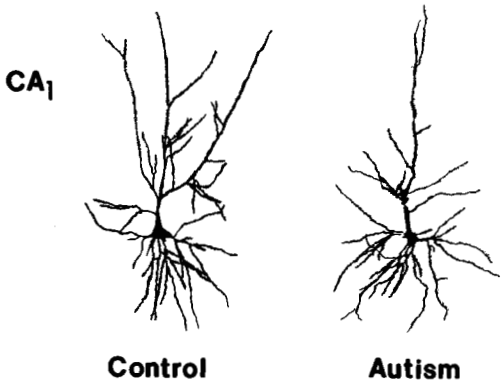

Fig. 1 Comparison of CA1 pyramidal neurons

Table 1 Perikaryon area (μm^2) of CA1 and CA4 pyramidal neurons

CA1 perikaryon area	
Autistic (n = 6)	Control (n = 5)
665.94	531.80
404.16	686.78
633.82	668.1
711.09	532.92
455.39	514.72
453.23	
CA4 perikaryon area	
Autistic (n = 5)	Control (n = 6)
584.33	753.63
500.11	675.49
594.75	694.60
558.28	645.10
522.25	798.35
	957.24

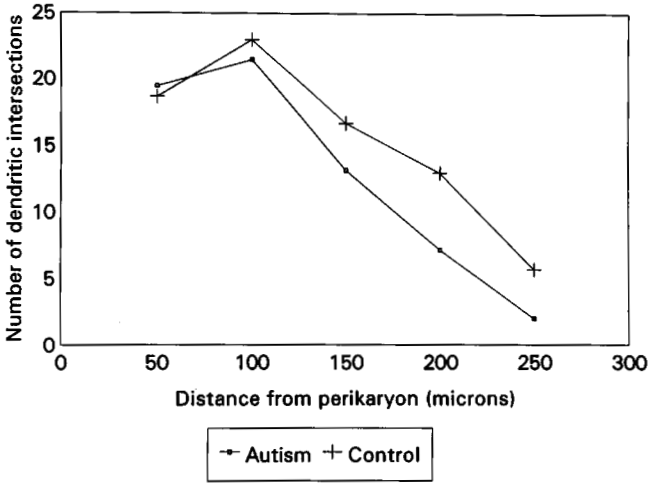

Fig. 2 Average dendritic intersections of concentric circles spaced 50 mm apart in CA4 neurons of the hippocampus in autistic (n = 5) and control (n = 6) children

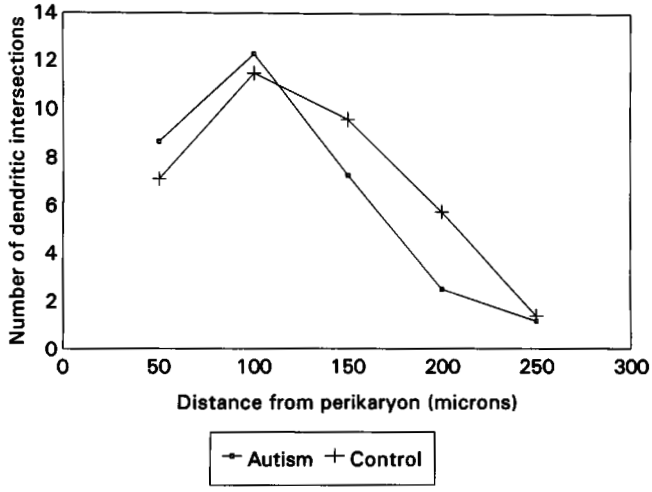

Fig. 3 Average dendritic intersections of concentric circles spaced 50 mm apart in CA1 neurons of the hippocampus in autistic (n = 6) and control (n = 5) children

Discussion

Involvement of the hippocampal complex may represent an important element in the atypical processing of certain types of information that is seen in individuals with autism [1]. The hippocampus has been suggested as a site for the primary lesion in autism [4, 7, 8] and some studies have associated acquired lesions of the hippocampus and medial temporal lobe to autistic-like behavior [5, 9, 10]. Clinical similarities between infantile autism and the Kluver-Bucy syndrome have also been noted [7]. However, hippocampal involvement alone may not be sufficient to account for all autistic behavior. Anatomic abnormalities in other limbic system structures have been reported [2, 3], as well as in the cerebellum [2, 3, 6, 12, 14], and there is growing evidence that these areas are important in memory, behavior, and learning [11].

We have demonstrated a decreased size of the neuronal perikaryon in CA4 neurons in one autistic child, and decreased complexity of dendritic branching in both CA4 and CA1 pyramidal cells in two autistic children in comparison with age-matched controls. Examination of the human brain with Golgi stain is a tenuous process. The quality of staining and the number of neurons stained are determined by factors that are not presently understood. Multiple determinants are believed to impact the staining process and specifically the quality of dendritic staining [16]. Sampling bias and other artifacts may also be important and further analysis of neuronal morphology in autistic and control brains should be pursued.

Our findings, however, are consistent with previous observations of reduced neuronal size and increased nerve cell packing density with routine stains [2]. Similar to the findings of Williams et al. [17] in the brains of individuals with autistic features, no dysmorphic neurons were found. The reduced cell size and simplified dendritic pattern seen in these autistic patients are consistent with a curtailment of maturation rather than malformation, where improperly aligned or disturbed cellular processes would be expected. Similar Golgi studies have shown decreased dendritic branching in neurons of the cerebral cortex in children with mental retardation [17]. This would suggest that failure of normal development at the cellular level may coincide with the functional status of the autistic individual. Although our findings are limited to two cases of infantile autism, these preliminary observations would support the need for further study of neuronal structure in additional regions of the limbic system as well as other areas of the hippocampal complex in this disorder.

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