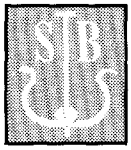

BRIEF REPORTS

Alterations in Hippocampal Mossy Fiber Pathway in Schizophrenia and Alzheimer's Disease

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Key Words: Dentate gyrus, entorhinal cortex, parahippocampus

Introduction

Schizophrenia and Alzheimer's disease (AD) both involve pathology of the medial temporal lobe. Structural abnormalities of the entorhinal cortex (Arnold et al 1991a; Arnold et al 1991b) and behavioral deficits implicating medial temporal circuits (Saykin et al 1991) have been reported in schizophrenia. The entorhinal cortex is the first and among the most severely effected regions in AD (Braak and Braak 1992). The entorhinal cortex receives input from higher-order multimodal and sensory-specific association cortices and transmits that information to the hippocampus via its connections with the dentate gyrus (Rosene an Van Hoesen 1987). The memory deficit in AD has been related to the disruption of this connection to the hippocampus, the perforant pathway (Hyman et al 1984). Despite the strong evidence of neuropathological changes in the entorhinal cortex in AD and schizophrenia, it is not known if they result in transneuronal changes of intrahippocampal connections. One method for testing this is to examine whether the density of mossy fibers, arising from the granule cells of the dentate gyrus and innervating the hilus, CA4 and CA3 subfields of the hippocampus (Rosene and Van Hoesen 1987), is altered. Experimentally induced unilateral entorhinal lesions lead to sprouting of the hippocampal mossy fibers (Steward 1992). Examination of the density of mossy fibers arising from the dentate granule cells has not been previously report4ed for these two diseases. We examined the staining intensity of the mossy fibers, following processing for Timm's histochemistry (Cassel and Brown 1984), in the hippocampus of schizophrenics, AD patients, and controls.

Methods

Blocks of brain tissues containing the medial temporal lobe, with the hippocampus included, were obtained from the Hospital of the University of Pennsylvania (Dr. J.Q. Trojanowski, Director of Medical Pathology, Hospital of the University of Pennsylvania) and from the Sun Health Research Institute (Dr. Joe Rogers, Director, Sun City, Arizona). The tissues included 11 cases with the clinical diagnosis of schizophrenia, nine cases with AD, and eight controls. The subjects were matched as closely as possible with respect to age, gender and times of post-mortem interval (PMI). The mean age ± SE of the schizophrenic patients was 76.5 ± 1.2 yr (range, 71–83 yr); AD patients, 79.9 ± 2.5 yr (range, 66–89 yr); and controls 69.0 ± 4.4 yr (range, 54–94 yrs). The PMI of schizophrenic (12.6 ± 1.5 hr), AD (7.8 ± 2.9 hr), and control (9.1 ± 2.4 hr) groups showed no differences ($F = 1.849, p = .1782$).

All patients with schizophrenia had been chronically institutionalized with a primary psychiatric diagnosis of schizophrenia confirmed by application of DSM-III-R criteria to clinical symptoms documented in hospital records (American Psychiatric Association 1987; Arnold et al 1994). AD cases were diagnosed according to DSM-III-R criteria for primary degenerative dementia and autopsy findings (neuropathologist: J.Q. Trojanowski, M.D.) of senile plaques and neurofibrillary tangles in both cerebral cortex and hippocampus above age-matched levels according to the guidelines of Khachaturian (1985). Alzheimer disease was ruled out for all schizophrenic and control cases using the same methods and criteria.

The midbody region of the hippocampus from all cases was sectioned at twenty microns, stored at −70°C, and processed in one

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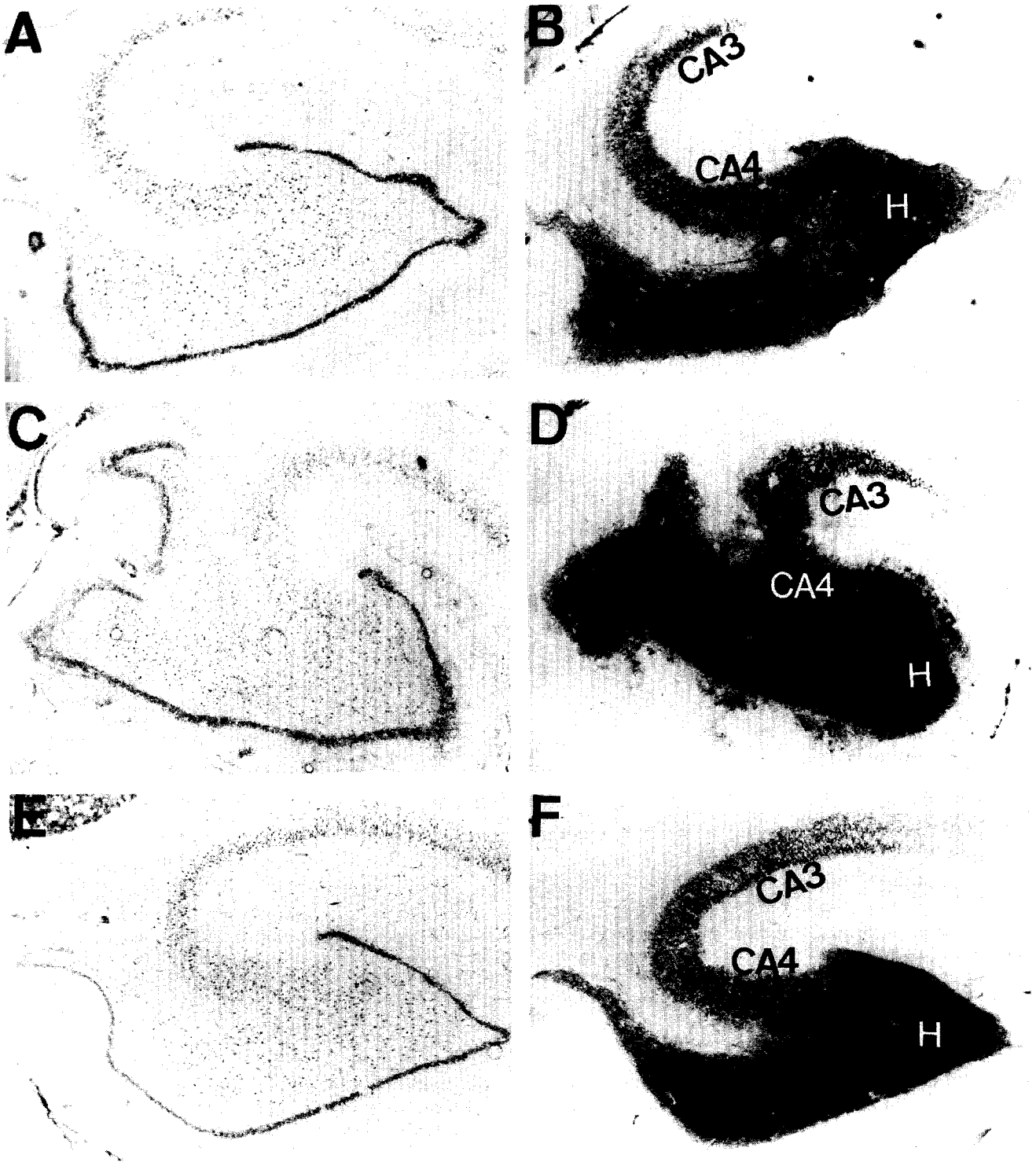

Figure 1. Timm's staining of mossy fibers was examined in schizophrenic (B), Alzheimer's disease (D), and control (F) cases. Histological identification of the hilus (H), CA4, and CA3 subfields of hippocampus were made in Nissl-stained material (A,C,E).

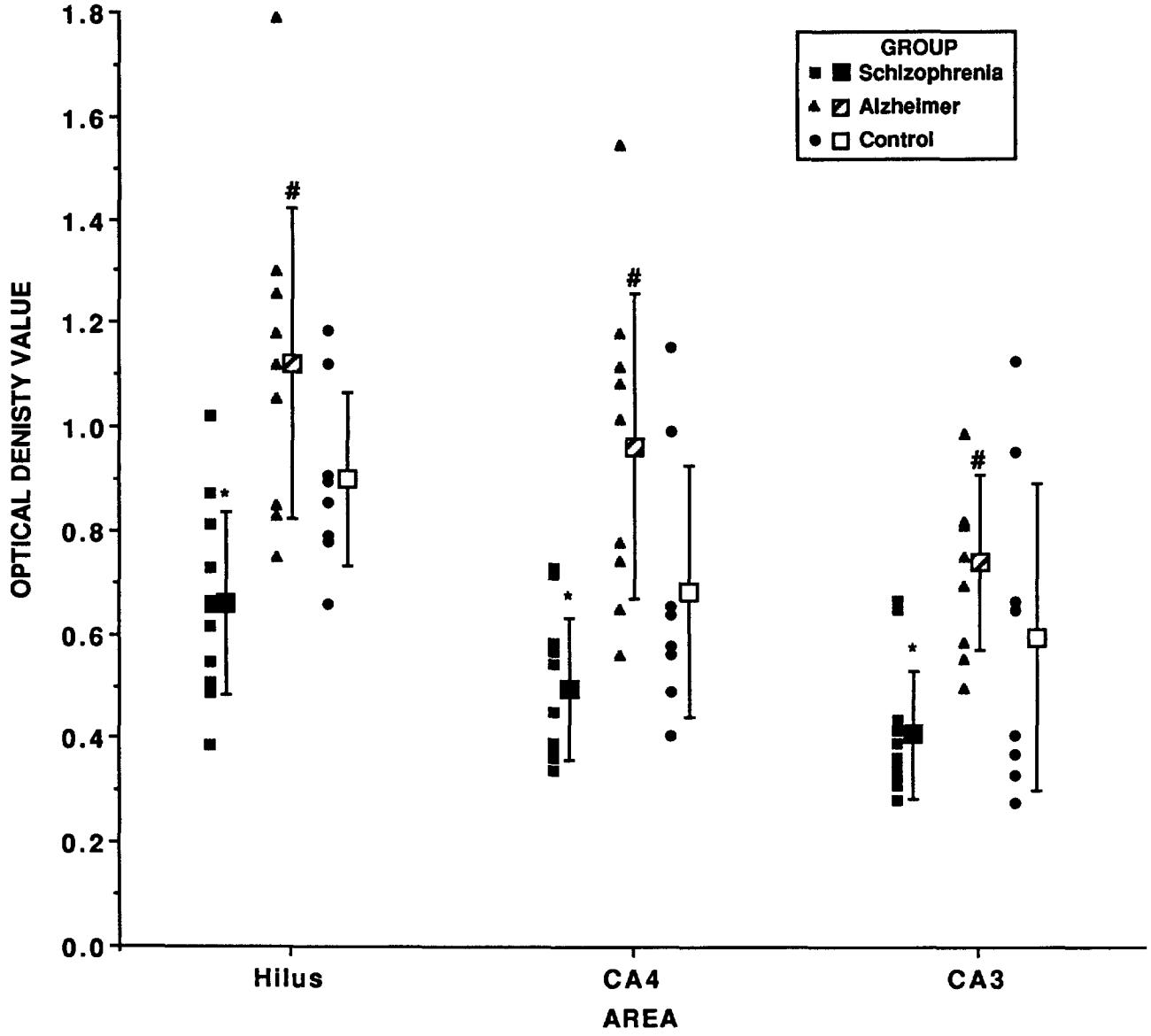

Figure 2. Graphs representing the mean ± standard error (*box with bar*) and the individual values for each subject (*circle, square, or triangle*) for the optical density readings for schizophrenia (*square*), Alzheimer's disease (*triangle*), and control groups (*circle*) are shown. Significant differences between schizophrenic and the Alzheimer's disease (*, $P < 0.0001$) and control groups (*, $P < 0.05$) are shown. Significant differences between Alzheimer's and control groups are shown (#, $P < 0.05$).

assay for Timm's stain for zinc (Cassell and Brown 1984) with slight modifications (Goldsmith and Joyce 1994; Ryoo and Joyce 1994). Alternate sections were stained with Nissl's Density of Timm's stain was quantified by two independent observers who were blind to the case identifier, and the analysis was from at least three sections from each case. An average value was determined for each case. Light transmission through ammonic subregions (hilus, CA4, CA3) was converted to an optical density value, based on comparison with a calibrated step tablet (Kodak), using a Macintosh-based image analysis (software package BRAIN:Drexel University, Philadelphia, PA). Differences in optical density between groups (regions as repeated measure) were determined by repeated measures analysis of covariance (ANCOVA) with age and PMI as covariates. Post-hoc comparisons were tested for significance of difference using the Scheffe's S test at the 95% confidence level.

Results

Timm's staining of mossy fibers was densest in the hilus of the dentate gyrus, with lighter staining in the CA4 and CA3 subfields

(Figure 1F). The staining intensity for the mossy fibers was lighter in all regions of hippocampus in the schizophrenic cases as compared to control or AD cases (Figure 1B; Figure 2). The AD cases showed higher staining than did control cases (Figure 1D; Figure 2). There were significant group ($F = 22.366$, $F = .0001$) and region ($F = 12.807$, $P = .0001$) effects. There was no group by region interaction ($F = 0.326$, $P = .857$), effect of age ($F = .135$, $P = .7148$), or effect of PMI ($F = .044$, $P = .8340$) on optical density values. The mean optical density value ± SD for the hilus (0.662 ± 0.18), CA4 (0.494 ± 0.142), and CA3 (0.408 ± 0.130) regions of the schizophrenic group were significantly lower than control ($P = .0049$) and AD group ($P = .0001$). The mean optical density value ± SD for the hilus (0.899 ± 0.17), CA4 (0.684 ± 0.25), and CA3 (0.598 ± 0.309) regions of the control group were significantly lower than the comparable regions (1.122 ± 0.316; 0.961 ± 0.31; 0.739 ± 0.178, respectively) of the AD group ($P = .00049$).

Discussion

This is the first report of changes in Timm's staining of hippocampal mossy fibers in AD and schizophrenia. The increase in staining of mossy fibers in AD is similar to that observed in animals after perforant pathway lesions (Frotscher et al 1994; Steward 1992), and may specifically reflect an increase in fiber density following hippocampal deafferentation in both cases. Alternatively, it may reflect an increase in staining per fiber. An overexpression of copper-zinc superoxide dismutase (SOD-1) has been suggested to be a cause of AD-related pathology (Epstein 1983; Perrin et al 1990). Mice transgenic for, and expressing the human SOD-1 gene exhibit reduced Timm's staining of hippocampal mossy fibers (Barkats et al 1993). Thus, the present finding of an increase in staining in AD does not support the proposal that overexpression of the SOD-1 gene models AD-related pathology.

The observed reduction in the staining intensity of the mossy fiber pathway in the schizophrenic group was unexpected and could reflect a reduction in the number of fibers or staining intensity per fiber. Reduced expression of other synaptic markers has been reported for the hippocampus of schizophrenics (Browning et al 1993). In agreement with the present findings, McLardy (1974, 1975) found reduced zinc per gram wet weight of hippocampus schizophrenics. Several converging lines of evidence have suggested the presence of deficits in the parahippocampus and hippocampus of schizophrenics. These include cytoarchitectural abnormalities, reduced expression of some cytoskeletal components of neurons, and modified expression of receptors (Arnold et al 1991a,b; Joyce 1993). Many of these abnormalities have been demonstrated in the entorhinal cortex, a major source of cortical input to the hippocampus. The abnormalities in the parahippocampus may occur during development (Suddath et al 1990). If so, this may result in permanent changes in the numbers of mossy fibers. Alternatively, the changes may reflect a response to chronic neuroleptic treatment acting through dopamine D2 receptors in the hippocampal complex (Goldsmith and Joyce 1994; Ryoo and Joyce 1994).

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