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ORIGINAL ARTICLE

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Intact hippocampal gray matter in schizophrenia as revealed by automatized image analysis postmortem

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Abstract Implicated as a key structure in the pathophysiology of schizophrenia, the hippocampus is at the forefront of neuropathological and neuroimaging research. To elucidate the cellular basis of hippocampal pathology in schizophrenia, we studied the postmortem hippocampal sections of 16 patients suffering from schizophrenia and 16 controls applying the gray-level index (GLI) method. We determined the area-percentage covered by neuronal perikarya in relation to the total area of the pyramidal cell layer in the four subdivisions of the ammon's horn (cornu ammonis, CA1–4) bilaterally. Additionally, we determined the area size of the pyramidal cell layer (CA1–4) and dentate gyrus (DG) granule cell layer. Results showed no significant differences between diagnostic groups with respect to the dependent variables, supporting the view that there is no primary alteration of hippocampal gray matter in schizophrenia.

Keywords Schizophrenia · Hippocampus · Postmortem · Morphometry

Introduction

Human hippocampal formation is critical for declarative memory (Scoville and Milner 1957), the dysfunction of which may impair reality testing and contribute to the schizophrenia phenotype. Substantial support for the hypothesis of hippocampal pathology in schizophrenia comes from in vivo neuroimaging research. Structural imaging studies have detected abnormalities of hippocampal size (meta-analytically reviewed in Lawrie and Abukmeil 1998; Nelson et al. 1998; Wright et al. 2000) and shape (Csernansky et al. 2002). A decrease in hippocampal volume (approximately 4% according to Nelson et al. 1998) is already present in subjects at risk (Lawrie et al. 1999; Pantelis et al. 2003) and patients with first-episode schizophrenia (Bogerts et al. 1990; Velakoulis et al. 1999), making it one of the most robust structural abnormalities in schizophrenia (Heckers and Konradi 2002). Hippocampal volume is not reduced in bipolar disorder (Altshuler et al. 2000) and schizotypal personality disorder (Dickey et al. 2002), indicating that this parameter is of high diagnostic specificity and may represent an endophenotype for schizophrenia (Gottesman and Gould 2003; Zobel and Maier 2004).

Functional imaging studies of schizophrenia have identified altered hippocampal activity in relation to psychotic symptoms (Friston et al. 1992), attentional deficits, and declarative memory impairment (e.g., Heckers et al. 1998; Jessen et al. 2003; Weiss et al. 2004). Proton magnetic resonance spectroscopy (^{1}H-MRS) has revealed a decrement of hippocampal *N*-acetyl aspartate (NAA) signal in schizophrenia, hence implicating a neuronal pathology (e.g., Bertolino et al. 1998).

By contrast, despite the plethora of postmortem morphometric studies, no consensus has yet evolved on the presence or nature of cytoarchitectonic abnormalities corresponding to hippocampal dysfunction in schizophrenia (Dwork 1997). Neuropathological findings of the altered morphology of the hippocampus and its neuronal organization, including presynaptic and dendritic parameters, suggest disturbances of functional circuitry within the hippocampus and its extrinsic connections particularly to the prefrontal cortex (reviewed by Harrison 2004).

To address the question of whether a decrease in hippocampal volume (Bogerts et al. 1985; Lawrie and

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Abukmeil 1998; Nelson et al. 1998; Wright et al. 2000) might result from gray matter changes, we studied postmortem hippocampal sections of 16 patients suffering from schizophrenia and 16 controls, applying the gray-level index (GLI) method. We determined the area-percentage covered by neuronal cell perikarya in relation to the total area of the pyramidal cell layer in each of the four subdivisions of the ammon's horn bilaterally. In addition, we determined the area sizes of the pyramidal cell layer (CA1–4) and granule cell layer (DG).

Material and methods

Brain collection

We studied the hippocampal formation in the brains of 16 patients suffering from schizophrenia (eight females; mean age: 53.3 ± 7.5 years; duration of illness: 21.9 ± 9.8 years) compared to the brains of 16 control subjects (eight females; mean age: 55.7 ± 10.5 years) collected between 1985 and 1995. A detailed survey of descriptive statistics of intervening variables (brain weight, postmortem delay, and formalin time) of the brain series has been given by Vogeley et al. (2003). Before autopsy, informed consent was obtained from the nearest relative or from responsible authorities in cases under legal care. Study procedures were approved by the local ethics committee of the Heinrich-Heine-University in Duesseldorf. Patients with systemic illness, alcohol or drug abuse disorders were excluded. At the time of death, most patients were treated with antipsychotic medication; total dosage over the last 6 months of life is available for all patients (Vogeley et al. 2003). Schizophrenia was diagnosed by two experienced psychiatrists according to DSM-III-R and ICD-9 criteria.

Staining and image acquisition

After formalin fixation, the tissue was embedded in paraffin and serial coronal sections perpendicular to the anterior–posterior commissure line, 20 μm in thickness, were cut using the same microtome under identical environmental conditions as a control for variance in section thickness. Modified Gallyas silver staining (Merker 1983) was performed on every 50th slice. From each subject, one section on the level of the lateral geniculate body was taken to create microscopic images of the hippocampal formation that were blinded with respect to subject and diagnosis. Images were acquired as digitized gray level (8 Bit) raw images with a CCD camera (SONY®) attached to a light microscope (Zeiss Planpro® 6.3×1.25, Germany) with a lens magnification of 10. The brightness was controlled by the histogram of the gray tones in the images, and adjusted to comparable values in all cases. Video frames corresponded to microscopic fields of approximately 1250×986 μm in size as assessed by a calibration standard. Adjoining video frames were obtained automatically using a computer-driven scanning table (Maerzhaeuser®, Wetzlar, Germany). An expanded digitized image assembled from approximately 100 adjoining frames was reconstructed semi-automatically and entered into further analyses (Optimas 6.0). According to cytoarchitectonic criteria (West and Gundersen 1990; Arnold 2000), we manually delineated the outlines of (i) the pyramidal cell layer in each ammonic segment (CA1–4) and (ii) the granule cell layer (DG). These five outlines per case were used to produce mask images which were postprocessed and analyzed as follows.

Image processing and GLI measurement

A grid of measurement fields (60×60 μm) was generated covering the regions of interest. All image-processing steps as described were performed in each and every measurement field separately (OPTIMAS 6.0): First, a smoothing filter (median filter 3×3) was applied to correct for local inhomogeneities of the gray value distribution in the measuring field. Second, a local threshold was obtained at the local minimum of the typically bimodal gray value frequency histogram according to which gray level images were binarized. Third, a binary erosion operation was performed in three cycles followed by two dilation operations to cut apparent connections between adjacent cells. Fourth, a filling operation was used to correct for holes in the area of the perikarya profiles (Wree et al. 1982). Fifth, the area percentage of perikarya was calculated and averaged across all measurement fields of one particular subregion. The dependent measure of the GLI gives the area-percentage covered by stained perikarya in the subregions. The DG was segmented employing an adaptive threshold after spatial filtering (median filter 3×3). As DG granule cells are densely packed, the GLI was not expected to be informative and remained undetermined for this region. Therefore, only the area covered by stained granule cell perikarya was measured and entered into statistical analysis. Image processing steps are shown in Fig. 1.

Statistical analysis

The GLI signal obtained for the pyramidal cell layer of each ammonic subfield (CA1–4) as well as the area sizes of the pyramidal cell layer (CA1–4) and granule cell layer (DG) were used as dependent variables. As assessed by correlation analyses, none of the intervening variables described by Vogeley et al. (2003) was identified as a confounding factor and therefore not considered as a covariate in statistical testing. The statistical analyses of the diagnostic effect on the dependent variables were performed with repeated measure analyses of variance (ANOVAs), one for each parameter, GLI (CA1–4) and area size (CA1–4 and DG). Subfield and

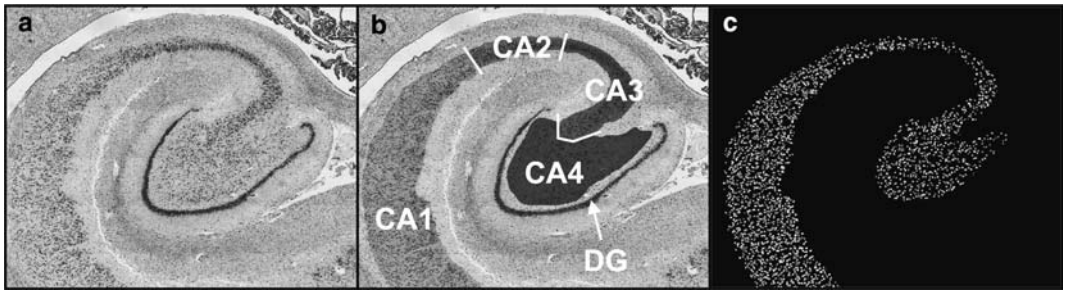

Fig. 1 **a** Coronal cross-section of the hippocampal body **b** illustrating the histological delineation of the ammonic subfields CA1–4 (pyramidal cell layer) and dentate gyrus (granule cell layer). **c** Typical aspect after image postprocessing (masking and binarization). The gray-level index (*GLI*) provides the area-percentage covered by stained perikarya (*white*) relative to the total area of the measurement field. *CA1–4* cornu ammonis segments 1–4 (pyramidal cell layer), *DG* dentate gyrus (granule cell layer)

side were within-subjects factors; diagnosis was entered as a between-subjects factor. As schizophrenia might affect hemispheres and hippocampal subfields differentially, we tested for interactions of diagnosis × side and diagnosis × subfield.

Results

The ANOVAs revealed neither a significant effect of diagnosis with respect to both area-percentage ($F_{1,30} < 1$, $p > 0.05$) and area size ($F_{1,30} < 1$, $p > 0.05$), nor significant diagnosis × side and diagnosis × subfield interactions. Neuron densities as reflected by the GLI as well as area sizes (mm^2) of the pyramidal cell layer (CA1–4) and granule cell layer (DG) are given in Table 1.

Discussion

The applied automatized image analysis method served as a scanning tool to investigate whether any pathological processes affecting hippocampal gray matter (pyramidal cell layer and DG granule cell layer) are present in schizophrenia. The main finding of this study is the absence of gray matter changes in the hippocampus in schizophrenia. However, as only coronal hippocampal sections at the level of the lateral geniculate body were assessed, abnormalities localized in the functionally distinct more anterior or posterior compartments along the rostrocaudal hippocampal axis (Strange et al. 2005) might have escaped verification. Since the lateral geniculate body has a spatial extent of up to several millimeters, we cannot rule out the possibility of histological variation across hippocampal sections as a confounding factor. Furthermore, the orientation of the studied hippocampal sections may slightly vary across brains. In addition, the GLI data might vary due to differences in irradiated brightness as the structures depicted in the reference images are not identical across specimens. To compensate for this source of variability, binary images, based on a local threshold given by the minimum of a bimodal gray level histogram, were produced for segmentation. Note that minor differences in lighting may influence the mean value of a gray level histogram, but leave the histogram distribution and the proportion of bright and dark areas unaffected.

Although a loss of hippocampal neurons in schizophrenia is discussed in the literature, only two studies have reported decreases in neuron density (Jeste and Lohr 1989; Jonsson et al. 1997). By contrast, several studies failed to detect abnormal neuron densities (Benes et al. 1991; Arnold et al. 1995), and one study revealed a right-sided increase (Zaidel et al. 1997a). The observed discrepancies might result from normal variation in the cytoarchitecture of the hippocampal formation, methodological restrictions including case selection, and differential influence of subfield or hemisphere on hippocampal pathology in schizophrenia (Dwork 1997). Single findings of an altered neuronal density restricted to a specific neuronal subtype (Benes et al. 1998), subfield or hemisphere (Zaidel et al. 1997b) deserve further investigation. The fact that existing stereological studies of the hippocampus in schizophrenia detected no difference in pyramidal cell density in any hippocampal subfield (Heckers et al. 1991; Walker et al. 2002; Highley et al. 2003) supports our conclusion that decreased hippocampal volume in schizophrenia is not due to selective gray matter changes but instead might arise from reduced hippocampal white matter (stratum oriens, stratum radiatum/lacunosum/moleculare) (Heckers et al. 1991; Heckers and Konradi 2002).

We addressed the question of regional specificity of changes in schizophrenia by dividing the hippocampus into five subfields which differ with respect to their cytoarchitecture, function, and susceptibility to schizophrenia (Harrison 2004). For instance, CA1—although vulnerable in Alzheimer's disease (West et al. 1994) and hypoxic brain injury (Petito et al. 1987)—is less affected in schizophrenia, concerning dysregulation of synaptic proteins and neurotransmitter receptors, while CA4 shows the reverse profile (Harrison 2004). However, consistent with stereological studies, we found no significant interaction of the factors diagnosis and subfield

Table 1 Neuron densities (reflected by the gray-level index, *GLI*) as well as area sizes (mm^2) of the pyramidal cell layer (CA1–4) and dentate gyrus (DG) granule cell layer as determined in postmortem hippocampal sections of 16 patients with schizophrenia and 16 controls

Subfield	Hemisphere	Neuron Density (GLI)				Area Size (mm^2)			
		Schizophrenic patients		Control subjects		Schizophrenic patients		Control subjects	
		Mean	SD	Mean	SD	Mean	SD	Mean	SD
CA1	Left	13.06	3.35	13.69	1.90	6.76	2.67	5.92	2.59
	Right	12.52	1.18	12.79	2.84	7.58	2.11	7.47	2.17
CA2	Left	12.29	3.38	13.12	1.43	0.70	0.19	0.76	0.25
	Right	11.85	2.26	12.14	2.81	0.68	0.34	0.77	0.21
CA3	Left	12.20	3.73	13.06	2.26	1.44	0.62	1.08	0.33
	Right	11.93	2.43	12.28	2.01	1.38	0.61	1.21	0.28
CA4	Left	12.52	3.07	13.65	2.03	2.39	0.60	3.49	1.43
	Right	12.36	3.05	12.85	1.98	2.85	0.16	2.82	0.87
DG	Left					0.74	0.21	0.97	0.33
	Right					0.75	0.22	0.92	0.30

SD standard deviation, *CA1–4* cornu ammonis segments 1–4 (pyramidal cell layer), *DG* dentate gyrus (granule cell layer)
Statistical analyses revealed no effect of schizophrenia

in our study, implying that a potential regional weighting of neurochemical anomalies is not necessarily reflected by morphometric indices.

The present study has been performed on a series of postmortem brains on which reports have already been published regarding schizophrenia-related cytoarchitectonic abnormalities in Brodmann area 10 (Kawasaki et al. 2000; Vogeley et al. 2003). In these studies, disease-specific disturbances were detected that were absent in the present study, suggesting that the hippocampus proper might be less susceptible to disturbances of neuronal composition than adjacent cortices in schizophrenia. For instance, morphometric studies of the entorhinal cortex in schizophrenia detected misplaced and aberrantly clustered pre-alpha cells—a cell type that gives rise to the perforant pathway, the major excitatory input to the hippocampus (Jakob and Beckmann 1986; Falkai et al. 2000). Such dysplastic changes might compromise hippocampal input and output pathways in schizophrenia, compatible with recent findings of hippocampal GABA(A) and AMPA/kainate glutamate receptor dysregulation in schizophrenia (reviewed by Heckers and Konradi 2002).

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