Eur Arch Psychiatry Clin Neurosci (1999) 249 : 169–173

ORIGINAL PAPER

Sven A. T. Jönsson · Anders Luts
Niels Guldberg-Kjaer · Rolf Öhman

Pyramidal neuron size in the hippocampus of schizophrenics correlates with total cell count and degree of cell disarray

Received: 26 November 1997 / Accepted: 20 January 1999

Abstract Hippocampal pyramidal neuron size was determined in all Cornu Ammonis subregions – CA1–CA4 – in five chronic schizophrenic men and compared with eight controls matched with respect to age and sex. Four out of five probands and the same eight controls had been examined in a previous study showing a significantly lower cell count and disorientation of pyramidal cells in the CA1–CA3 subregions of the schizophrenics. There was also a negative correlation between the total number of cells and the number of disoriented cells. In this study it was shown that the schizophrenic probands also had significantly smaller neurons in all subregions. There was a significant negative correlation between pyramidal neuron size and the number of disarrayed neurons in each subregion, and there was a significant positive correlation between neuron size and the total number of pyramidal cells in CA1 and CA2, but not in CA3 and CA4. The consistency of hippocampal anomalies in these schizophrenics is, thus, demonstrated by the statistical relations between the different parameters examined.

Key words Schizophrenia · Hippocampus · Pyramidal cell size · Cell count · Cell disarray

Introduction

Considerable morphometric evidence has been accumulated in recent years indicating abnormalities in the hippocampus of schizophrenics plausibly of relevance for the pathophysiology of this disease [for reviews see 3, 14]. Findings include a decrease in volume [6–8, 21], a decrease in neuron density [16, 21], and a decrease in neuron size [3, 5, 31] as well as a disarray in the pattern of pyramidal cell organization [13, 23, 26]. As to connectivity, indications of a decrease have been reported both in studies employing staining techniques [17] and immunochemical probes for synapse-related proteins and mRNA for such proteins [9, 15]. A recent finding by Harrison and Eastwood [18] of a lower expression of presynaptic complexin proteins in the hippocampus of schizophrenics implicates a reduced arborization of dendritic connections assumed to be associated with smaller cell bodies. Their study does, however, not contain any information on cell size.

With respect to most of these parameters there are, however, reports failing to confirm abnormalities. There are several studies not confirming a volumetric decrease [1, 5, 10, 12, 19, 20] and the same is true about neuron density [5, 20, 23] and neuron size [11]. Findings on connectivity and dendritic arborization are still few and so are replicative efforts. As to the pyramidal cell disarray first reported by Scheibel and Kovelman in 1980, several attempts to replicate their findings have failed [2, 3, 5, 11, 20, 31] although data sometimes suggest a bimodal distribution of cases with respect to this parameter. The degree of pyramidal cell disarray has been associated with severity of illness [2] or subdiagnosis; aberrations in the normal patterns of cell organization being more apparent in patients with paranoid schizophrenia than in catatonics and hebephrenics [23]. In a recent study, the present authors could demonstrate that there was a significant disarray of pyramidal cells in Cornu Ammonis subregions CA1–CA3 in a small cohort of chronic schizophrenic men. These patients also had significantly fewer neurons than age-matched controls in CA1–CA3, but not in CA4 or in the granular cell layer of the dentate gyrus. There was no reactive gliosis. In all subregions CA1–CA3 there was a significant negative correlation between the total number of observed pyramidal cells and the number of disarrayed cells [22]. In a further study it could be shown that these abnormalities were remarkably uniform in a pairwise comparison with normal age-matched controls [24]. In the present study the size of pyramidal cells has been determined in samples from subregions CA1–CA4.

S. A. T. Jönsson (✉) · A. Luts · N. Guldberg-Kjaer · R. Öhman
Department of Clinical Neuroscience, Division of Psychiatry,
Lund University Hospital, S-22185 Lund, Sweden
e-mail: Sven.Jonsson@psykiatr.lu.se

Material and methods

Probands

For details see Jönsson et al., 1997, Probands 1–4 were included in a previous study [22] and proband 5 (5/317/84) has been added. Therapeutic response was poor in all probands and they were, therefore, cared for at a ward for chronic cases. Mean age at death was 81.8 ± 2.6 years. Briefly the following case descriptions are given.

Proband 1/408/79 (proband no/brain no/age at death). First ill at 23 years. Symptom picture included auditory hallucinations and delusions. Aggressive and agitated. Ritualistic behavior. Periodically mute. Lobotomy at the age of 35. Diagnosis: Catatonic schizophrenia, 295.2. Cause of death: Cardiac insufficiency.

Proband 2/384/79. Probably an insidious disease process. First hospitalized at the age of 44. Auditory hallucinations and persecutory delusions. Aggressive and threatening. Considered dangerous. Diagnosis: Paranoid schizophrenia, 295.3. Cause of death: Bronchopneumonia.

Proband 3/332/80. Hospitalized at the age of 28. Auditory hallucinations and persecutory delusions. Periodically irritable and aggressive. Disorganized behavior. Diagnosis: Paranoid schizophrenia, 295.3. Cause of death: Cardiac infarction.

Proband 4/302/81. First ill of depressive syndrome at the age of 27. Symptom-free after a few months. Inconspicuous and at work until the age of 38. Negativistic, mutistic, nihilistic delusions, and catatonic posturing. Never recovered. Diagnosis: Catatonic schizophrenia, 295.2. Cause of death: Pneumonia.

Proband 5/317/84. Fell ill already at the age of 17. Aggressive and assaultive. Rapid deterioration during the first year of illness. Thereafter practically mute throughout life. Probably hallucinations. Profoundly blunted affect and exacerbations of aggressiveness every year. Diagnosis: Catatonic schizophrenia, 295.2. Cause of death: Volvulus with gut gangrene and peritonitis.

Controls

The brains from eight age-matched male controls dying from non-cerebral diseases and without known mental illness were obtained as control cases from the Department of Pathology at University of Lund. Mean age at death was 76.5 ± 8.5 years. These brains had been neurohistologically examined and no gross pathology had been detected. For details concerning the controls see Table 1.

Table 1 Control subjects; causes of death

Case No/Brain No/ Age at death	Causes of death
1/232/85	Cardiac infarction and pulmonary embolism
2/159/74	Aortic aneurysm rupture
3/621/72	Pancreatic cancer
4/274/81	Pulmonary embolism and ileus
5/248/85	Bronchopneumonia and cardiac insufficiency
6/689/82	Cardiac infarction and insufficiency
7/446/73	Cardiac infarction
8/467/60	Cardiac infarction

Tissue handling

For details see Jönsson et al. 1997 [22]. Briefly, the brains were fixed in 4% formaldehyde, never later than 24 h post mortem and examined grossly. Five μm sections from mid hippocampus of the left hemisphere were provided and stained with hematoxylin eosin, cresyl violet, and Luxol fast blue.

Gross and microscopic examination

In the control cases no pathological changes were disclosed but alterations pertaining to normal aging. Similar changes were noted in the brains of probands. No signs of gross vascular diseases or tumors were detected. There were small infarctions in different areas of the cortex, as generally seen in elderly people, but not in the region here examined. In several probands changes as slightly widened ventricles were seen, but in none of them were there signs of major age-related degenerative disease. One of the probands (4/302/81) had a congenital malformation, a mild cortical dysplasia of the frontobasal cortex on one side, and another proband (5/317/84) had a gliotic sclerosis in the right hippocampus, probably indicating a cerebral infarction. There were no significant differences in the mean number of glial cells in any of the CA-subsections.

Morphometric analysis

The sections were coded and the morphometric analyses were performed by one investigator with no access to diagnosis. The Cornu Ammonis sectors CA1–CA4 were identified (see 21). One visual field located in the center of every CA sector in each section was chosen. The number of all pyramidal cells, i.e., including those in which the nucleus could not be recognized, was determined, and the mean area of these cells in each visual field of the different CA sectors was calculated using the Quantimet Q500 MC image analysis system (Leica Cambridge Ltd, Cambridge, UK). Pyramidal cell orientation was measured in samples containing 100 cells counted from the centers of each subfield CA1–CA3. The number of cells deviating more than 15°, 25°, and 35° from expected perpendicular position was determined. The pyramidal cells of CA4 lack the unequivocally perpendicular orientation seen in CA1–CA3; this subregion was, therefore, not examined with respect to cell disarray.

Statistics

In group comparisons Mann-Whitney U-test was used and in correlation analyses Spearman's rank order correlation coefficient.

Results

In all subregions pyramidal cells were significantly smaller than in the controls (see Table 2 and Fig. 1).

Table 2 Mean area of pyramidal cells in CA1, CA2, CA3, and CA4 (μm^2)

Region	Schizophrenic probands (N = 5)	Controls (N = 8)	Z	P
CA1	212.7 ± 24.0	353.4 ± 31.6	–2.93	0.0034
CA2	254.3 ± 25.3	415.0 ± 88.9	–2.93	0.0034
CA3	254.2 ± 71.4	457.4 ± 115.9	–2.63	0.0084
CA4	271.9 ± 71.9	523.5 ± 106.9	–2.93	0.0034

Mann-Whitney U-test

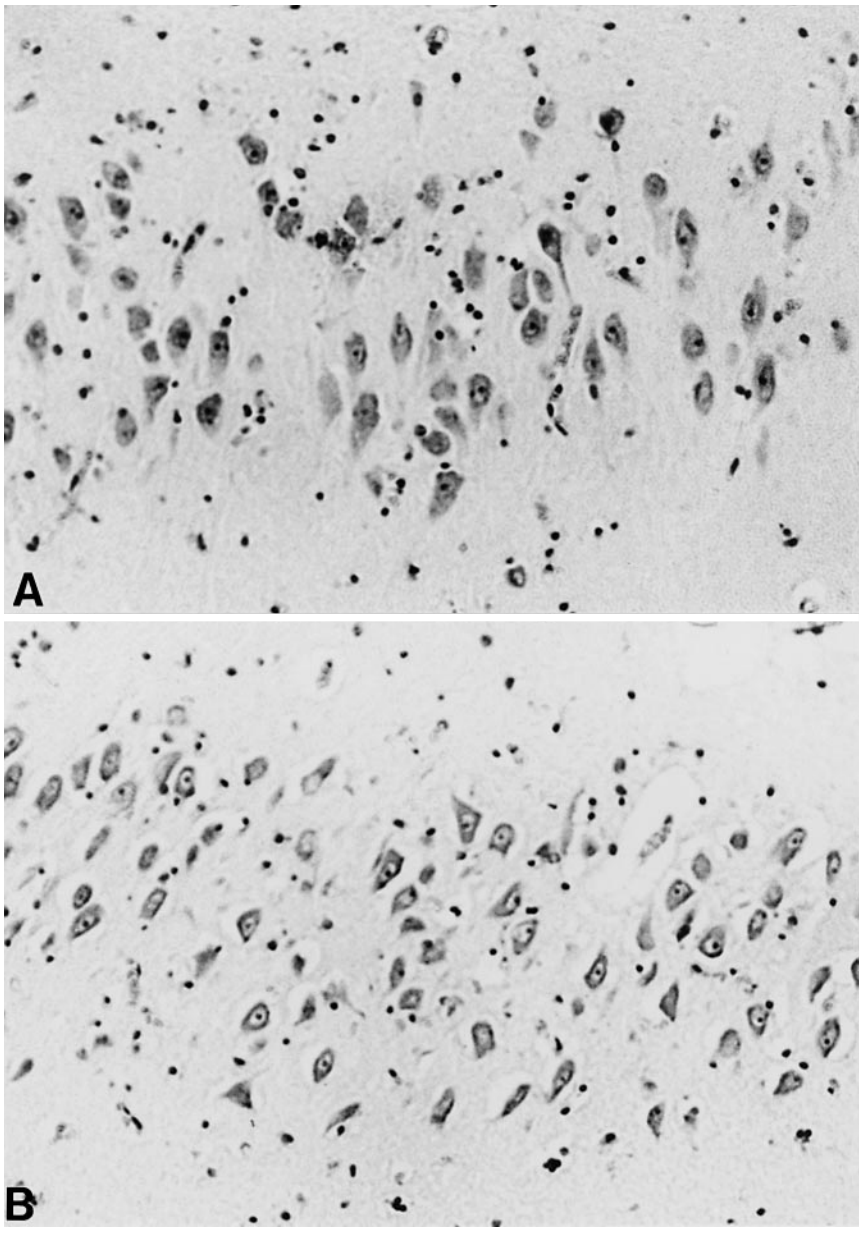

Fig. 1 Sections from CA1 demonstrating the difference in pyramidal cell size and disarray. A. control, B. schizophrenic proband. × 400

Table 3 Correlations between the number of deviating cells per 100 cells and the mean area of pyramidal cells in respective region. Schizophrenic probands and controls, N = 13

Region and degree of deviation	r_s	P
CA1; > 35°	–0.715	0.006
CA1; > 25°	–0.690	0.009
CA1; > 15°	–0.707	0.007
CA2; > 35°	–0.721	0.005
CA2; > 25°	–0.740	0.004
CA2; > 15°	–0.857	0.000
CA3; > 35°	–0.843	0.000
CA3; > 25°	–0.642	0.018
CA3; > 15°	–0.632	0.021

Spearman's rank-order correlation

Table 4 Correlations between the total number of pyramidal cells and the mean area of pyramidal cells in CA1–4. Schizophrenic probands and controls (N = 13)

Region and degree of deviation	r_s	P
CA1	0.593	0.033
CA2	0.637	0.019
CA3	0.385	NS
CA4	0.341	NS

Spearman's rank-order correlation

There was a significant negative correlation between the mean size of pyramidal cells and the number of these cells deviating more than 35°, 25°, and 15° from the normal perpendicular position in all subregions (see Table 3).

There was a significant positive correlation between the mean size of pyramidal cells and the total number of these cells in subregions CA1 and CA2, but not in CA3 and CA4 (see Table 4).

Discussion

Differences in the mean area of pyramidal neurons between studies may partly be explained by differences in morphometric methods – whether all cell fragments have been measured or only cells with a recognizable nucleus, for example. The figures here recorded are in the interval seen in other studies.

In the present study significant correlations between cell area and cell disarray were seen in subregions CA1–CA3 which give further strength to the differences seen between groups. This might actually be a geometrical artifact due to a reduction in the observed area of disarrayed cells when sectioned in another plane than the perpendicular position possibly showing a larger area. In such a case, the present finding confirms the observation that some schizophrenic patients have an excess of disarrayed cells. On the other hand, there are also significant correlations between the total number of cells in the observed areas in CA1 and CA2 which is an argument for the contention that the smaller cell area in schizophrenics is a genuine finding. These correlations are, however, not seen in CA3 and CA4, the latter subregion being the most vulnerable to age-dependent changes in terms of cell death [25, 28]. The schizophrenic probands are slightly older than the controls (81.8 ± 2.6 vs. 76.5 ± 8.5 years). Cell shrinkage as an effect of an age-related decline of cell function has been demonstrated in recent years, but cell number does not seem to be much affected by age in subfields CA1–3, when correcting for relevant factors [27, 29, 30].

Since the difference in age between probands and controls probably can be neglected, the statistical relations between different parameters here established support the conclusion that hippocampal pathology in schizophrenia may include a reduction in pyramidal cell number, a disarray of such cells, and a reduction of cell area. Still, there is a possibility that the latter finding may be explained as a geometrical artifact.

References

1. Altshuler LL, Casanova MF, Goldberg TE, Kleinman JE (1990) The hippocampus and parahippocampus in schizophrenic, suicide, and control brains. Arch Gen Psychiatry 47: 1029–1034
2. Altshuler LL, Conrad A, Kovelman JA, Scheibel A (1987) Hippocampal pyramidal cell orientation in schizophrenia. A controlled neurohistologic study of the Yakovlev collection. Arch Gen Psychiatry 44: 1094–1098
3. Arnold SE, Franz BR, Gur RC, Gur RE, Shapiro RM, Moberg PJ, Trojanowski JQ (1995) Smaller neuron size in schizophrenia in hippocampal subfields that mediate cortical-hippocampal interactions. Am J Psychiatry 152: 738–748
4. Arnold SE, Trojanowski JQ (1996) Recent advances in defining the neuropathology of schizophrenia. Acta Neuropathol 92: 217–231
5. Benes FM, Sorensen I, Bird ED (1991) Reduced neuronal size in posterior hippocampus of schizophrenic patients. Schizophr Bull 17: 97–608
6. Bogerts B, Meertz E, Schonfeldt-Bausch R (1985) Basal ganglia and limbic system pathology in schizophrenia. Arch Gen Psychiatry 42: 784–791
7. Bogerts B, Falkai P, Haupts M, Greve B, Ernst ST, Tapernon-Franz U, Heinzmann U (1990) Post-mortem volume measurements of limbic system and basal ganglia structures in chronic schizophrenics: Initial results from a new brain collection. Schizophr Res 3: 295–301
8. Bogerts B, Lieberman JA, Ashtari M, Bilder RM, Degreef G, Lerner G, Johns C, Masiar S (1993) Hippocampus-amygdala volumes and psychopathology in chronic schizophrenia. Biol Psychiatry 33: 236–246
9. Browning MD, Dudek EM, Rapier JL, Leonard S, Freedman R (1993) Significant reduction in synapsin but not synaptophysin specific activity in the brains of some schizophrenics. Biol Psychiatry 34: 529–535
10. Bruton CJ, Crow TJ, Frith CD, Jagoe K, Johnstone EC, Owens DGC, Roberts GW (1990) Schizophrenia and the brain: a prospective clinico-neuropathological study. Psychol Med 20: 285–304
11. Christison GW, Casanova MF, Weinberger DR, Rawlings R, Kleinman JE (1989) A quantitative investigation of hippocampal pyramidal cell size, shape and variability of orientation in schizophrenia. Arch Gen Psychiatry 46: 1027–1032
12. Colter N, Battal S, Crow TJ, Johnstone EC, Brown R, Bruton C (1987) White matter reduction in the parahippocampal gyrus of patients with schizophrenia. Arch Gen Psychiatry 44: 1023
13. Conrad AJ, Abebe T, Austin R, Forsythe S, Scheibel AB (1991) Hippocampal pyramidal cell disarray in schizophrenia as a bilateral phenomenon. Arch Gen Psychiatry 48: 413–417
14. Dwork AJ (1997) Postmortem studies of the hippocampal formation in schizophrenia. Schizophr Bull 23: 385–402
15. Eastwood SL, Burnet PWJ, Harrison PJ (1995) Altered synaptophysin expression as a marker of synaptic pathology in schizophrenia. Neuroscience 66: 309–319
16. Falkai P, Bogerts B (1986) Cell loss in the hippocampus of schizophrenics. Eur Arch Psychiatr Neurol Sci 236: 154–161
17. Goldsmith SK, Joyce JN (1995) Alterations in hippocampal mossy fiber pathway in schizophrenia and Alzheimer's disease. Biol Psychiatry 37: 122–126
18. Harrison PJ, Eastwood SL (1998) Preferential involvement of excitatory neurons in medial temporal lobe in schizophrenia. Lancet 352: 1669–1673
19. Heckers S, Heinsen H, Heinsen YC, Beckman H (1991) Limbic structures and lateral ventricle in schizophrenia: a quantitative postmortem study. Arch Gen Psychiatry 47: 1016–1022
20. Heckers S, Heinsen H, Geiger B, Beckmann H (1991) Hippocampal neuron number in schizophrenia. A stereological study. Arch Gen Psychiatry 48: 1002–1008
21. Jeste DV, Lohr JB (1989) Hippocampal pathologic findings in schizophrenia. A morphometric study. Arch Gen Psychiatry 46: 1019–1024
22. Jönsson SAT, Luts A, Guldberg-Kjaer N, Brun A (1997) Hippocampal pyramidal cell disarray correlates negatively to cell number: implications for the pathogenesis of schizophrenia. Eur Arch Psychiatry Clin Neurosci 247: 120–127
23. Kovelman JA, Scheibel AB (1984) A neurohistological correlate of schizophrenia. Biol Psychiatry 19: 1601–1621
24. Luts A, Jönsson SAT, Guldberg-Kjaer N, Brun A (1998) Uniform abnormalities in the hippocampus of five chronic schizophrenic men compared with age-matched controls. Acta Psychiatr Scand 98: 60–64
25. Mani RB, Lohr JB, Jeste DV (1986) Hippocampal pyramidal cells and aging in the human: A quantitative study of neuronal loss in sectors CA1 to CA4. Exp Neurol 94: 29–40

26. Scheibel AB, Kovelman JA (1981) Disorientation of the hippocampal pyramidal cell and its processes in the schizophrenic patient. Biol Psychiatry 16:101–102
27. Terry RD, DeTeresa R, Hansen LA (1987) Neocortical cell counts in normal human adult aging. Ann Neurol 21:530–539
28. West MJ (1993b) Regionally specific loss of neurons in the aging human hippocampus. Neurobiol Aging 14:287–293
29. Wickelgren I (1996a) Is hippocampal cell death a myth? Science 271:1229–1230
30. Wickelgren I (1996b) For the cortex, neuron loss may be less than thought. Science 273:48–50
31. Zaidel DW, Esiri MM, Harrison PJ (1997) Size, shape, and orientation of neurons in the left and right hippocampus: investigation of normal asymmetries and alterations in schizophrenia. Am J Psychiatry 154:812–818