Distribution of Parvalbumin-Immunoreactive Neurons in Brain Correlates with Hippocampal and Temporal Cortical Pathology in Creutzfeldt-Jakob Disease

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Abstract. There is a distinctive pattern of hippocampal involvement in Creutzfeldt-Jakob disease (CJD) and evidence for selective vulnerability of GABAergic neurons in experimental and human prion disease. We studied hippocampus and temporal cortex from human CJD and control autopsy brains and surgical cryosectional temporal lobe epilepsy specimens for distribution and density of parvalbumin (PV) and calbindin-D28K (Cal) -positive neurons that are subpopulations of GABAergic neurons. Pathology was evaluated semiquantitatively in 8 regions in 23 CJD brains for severity of spongiform change, astrogliosis and pathological prion protein deposition. In CJD, pathology was severe in pre- parasubiculum and temporal cortex, and little or absent in CA1-4; PV+ neurons were severely reduced or absent in all cases, whereas Cal+ neurons were largely preserved. In controls, the density of PV+ neurons was highest in pre-parasubiculum and temporal cortex, and lowest in CA1-4. In cTLE, loss of PV+ neurons was seen only in CA1-4. The diffuse and severe loss of PV+ neurons in CJD, and the topographical correlation of tissue lesioning in CJD with density of PV+ neurons in controls suggest selective vulnerability and early loss of this subset of inhibitory neurons in CJD. This might relate to characteristic CJD symptoms such as myoclonus and the distinctive EEG pattern.

Key Words: Calbindin; Creutzfeldt-Jakob disease; GABA; Hippocampus; Parvalbumin; Prion disease; Temporal lobe epilepsy.

INTRODUCTION

Creutzfeldt-Jakob disease (CJD) is the prototype of human transmissible spongiform encephalopathies (prion diseases) (1). Spongiform change (SC), astrogliosis (AG) and neuronal loss are the classical neuropathological triad of tissue lesioning in CJD (2); deposition of pathological PrP (PrPSc) in the CNS is the hallmark of prion disease (3). Little is known about the pathogenetic basis of characteristic symptoms in CJD such as myoclonus and characteristic EEG changes. Experimental data demonstrated preferential alteration of the inhibitory GABAergic system in prion disease and prion protein knockout mice (4–9). Parvalbumin-positive (PV+) and calbindin-D28K positive (Cal+) neurons are nonoverlapping subpopulations of GABAergic neurons (9, 10). The number of PV+ neurons is severely reduced in the cerebral cortex of CJD as compared with control brains; this loss was suggested as pathogenetic basis for symptoms observed in the early stage of disease (9).

The hippocampus is an unique anatomical structure composed of distinct neuronal populations (10, 11). Its preservation in CJD has been reported (12, 13), but has not been studied in detail in a large series. In hippocampi of CJD autopsy cases, we observed a distinctive pattern of pathological involvement of anatomical regions. We present here the correlation between neuronal subpopulations and tissue lesioning in CJD.

MATERIALS AND METHODS

Formal-fixed, paraffin-embedded human tissue was used. Twenty-six blocks containing hippocampus and adjacent temporal cortex from 23 neuropathologically confirmed CJD autopsy cases from a series reported previously (14) and 10 blocks from 10 control brains were studied. Surgical temporal lobe resection specimens from 3 cryosectional temporal lobe epilepsy (cTLE) cases were also used as controls. Age of CJD patients (11 females and 12 males) ranged from 45 to 82 years (mean = 63.5; median = 63); age in the control group (4 females and 6 males) ranged from 31 to 76 years (mean = 56.9; median = 61.5); age in the cTLE group (1 female and 2 males) ranged from 29 to 37 years (mean = 33.3; median = 34). In controls, cause of death was heart and circulation failure (2 cases), pulmonary embolism (2 cases), myocardial infarction (1 case), polytrauma (1 case), traumatic brain injury (1 case), metastasizing carcinoma (2 cases), and acute lymphoblastic leukaemia (1 case).

Paraffin sections for GFAP and PrP immunostaining were cut at 4 μm, and for parvalbumin and calbindin-D28K immunostaining at 25 μm. Staining for parvalbumin and calbindin-D28K was performed on dewaxed free-floating sections. Monoclonal antibodies against PrP (3F4, 1:2000, Senetek PLC, Maryland Heights, Missouri), calbindin-D28K (CL-300, 1:200, Sigma, St. Louis), and parvalbumin (PA-235, 1:5000, Sigma, St. Louis), and a polyclonal antibody against GFA (1:100, Dako, Glostrup, Denmark) were used. The sections for PrP labeling were pretreated as follows: hydrated autoclaving at 121°C for 30 minutes (min), incubation in 98% formic acid for 1 min, and incubation in guanidine thiocyanate at 4°C for 2 h. Monoclonal antibodies were visualized by the AEC technique, polyclonals by the PAP technique. Hematoxylin counterstaining was performed on sections for cell quantification.

Semiquantitative evaluation of pathology was made as follows: the 8 investigated regions (CA4, CA3, CA2, CA1, subiculum [Sub], pre-parasubiculum [PrS], entorhinal cortex [EC] and temporal cortex [TC]) were graded in every individual section from 1 (minimum) to 8 (maximum) concerning severity of
spongiform change (SC), astrogliosis (AG) and amount of pathological PrP deposition. Lack of SC, AG and PrP deposition was graded as zero, respectively. This grading reflects an individual scale of the topographical distribution of specific features in a given hippocampus. PV+ neurons were quantified in the same regions of control and CJD cases by counting PV+ cell bodies in several fields with a 20× objective. The field with maximal number of PV+ neurons in the respective region was entered into statistical evaluation.

The significance of the difference of mean regional densities of PV+ neurons in the CJD and control groups was tested by means of the Permutation-T-test. Only cases showing all 8 regions in one block (19/23 CJD cases, in 4 cases TC and/or EC and PrS were not present; 9/10 controls) were entered into statistical evaluation.

RESULTS

In 24/25 investigated CJD blocks, SC was less severe in CA1-4 than in the other regions; in 23/26 blocks, SC in CA1-4 was slight or absent. In 15/26 blocks, SC was most prominent in PrS and TC, moderate in Sub and EC, and slight or absent in CA1-4 (Figs. 1, 2). In 3/26 blocks, SC was prominent in PrS or TC; in other areas SC was slight or absent. In 4/26 blocks, SC was equally severe in TC, EC, and PrS, or slight or prominent in absent in CA1-4 and Sub. In 2/26 blocks, maximal SC occurred in Sub. In 1 block, maximal SC was detected in CA4. In another block, SC was absent. In all cases, AG was prominent in TC, EC and PrS, and slight or absent in Sub to CA4. In all blocks, PrP deposition was less prominent in CA1-4 than in the other regions. Thus, the topographical profile of SC and AG correlated with the extent of PrP deposition. The cTLE specimens showed neuronal loss and astrogliosis most prominently in CA1, moderately in CA3 and CA4, and least in CA2.

All controls had a similar profile of regionally differing density of PV+ neurons, with highest density in TC and PrS, moderate density in EC and Sub, and low density in CA1-4 (Figs. 1, 2). Regionally differing density of PV+ neurons was paralleled by intensity of neuropil staining for parvalbumin. In CJD, all investigated regions of most cases had a similar degree of severe loss of PV+ neurons (range of average loss: 85–92%, compared with the mean in controls) and lack of parvalbumin neuropil staining, irrespective of the local severity of pathologic changes (Figs. 1, 2). The differences between density of PV+ neurons in CJD and in control brains are significant (p = 0.00001, Permutation-T-test). The difference of density of PV+ neurons in CJD and in controls in every single region is also significant (p = 0.0001, corrected according to Bonferroni-Holm). In only a small fraction (5.9%) of the investigated regions of all blocks, loss of PV+ neurons was slight (<33%, compared with the mean in controls, in 1 to 4 regions per block). Still rarer, in 3.8% of the regions, no loss of PV+ neurons was seen at all. In cTLE, loss of PV+ neurons was seen only in areas CA1-4. Sub, PrS and TC appeared normal (Fig. 1). In none of our cTLE specimens was EC available or clearly identifiable. In all investigated groups (controls, CJD and cTLE) the morphology of the surviving neurons appeared normal (Fig. 3).

The regional profile of pathologic changes in CJD correlates well with the density profile of PV+ neurons in controls (Figs. 1, 2), as does the amount of PrP. Thus, regions with high density of PV+ neurons in controls are severely damaged in CJD, and regions with fewer PV+ neurons in controls harbor little pathology in CJD.

In controls, a profile of regionally differing density of Cal+ neurons was seen, with highest density in TC and CA3-4, moderate density in EC and Sub, and low density in CA1-2 and PrS. In CJD brains, Cal+ neurons were well preserved, even in regions with moderate spongiform change and total loss of PV+ neurons (Fig. 4).

![Fig. 2.](http://jnen.oxfordjournals.org/)

**Fig. 2.** Graphic representation of hippocampal and temporal cortical density of PV+ neurons in controls and CJD and severity of SC in CJD. The y axis on the left indicates the number of PV+ neurons, the y axis on the right shows the grade of SC in CJD (range of grades: 0 = lack of; 1 = minimum; 8 = maximum). The columns depict the average numbers of PV+ neurons in controls and CJD (bar = SEM), the curve shows the average grades of SC in CJD in the regions CA1-4, PrS (presubiculum), EC (entorhinal cortex) and TC (temporal cortex). Regionally different density of PV+ neurons in controls correlates with regional severity of SC in CJD.

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**Fig. 1.** H&E (A, B, C, G, H, I) and parvalbumin immunostaining (D, E, F, K, L, M) of CA4 (A–F) and temporal cortex (G–M) in a control (A, D, G, K) and CJD (B, E, H, L) and cTLE (C, F, I, M) brain. Regionally differential density of PV+ neurons of the control brain (D, K) correlates with differential severity of SC in CJD (B, H). Despite the "normal" appearance of the CA4 region (B) in the CJD brain, subtotal (only one PV+ cell was left in the whole CA4 region) loss of PV+ neurons and neuropil staining was observed (E). Original magnification: ×128.

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Their density was reduced only in regions with extremely severe neuronal loss and SC.

DISCUSSION

In brain disorders presenting with some symptoms shared also by CJD, such as excitatory amino acid intoxication, Alzheimer disease, and epilepsy, the hippocampus is severely damaged (15–19). In Huntington’s disease, status epilepticus, hypoglycemic and hypoxic-ischemic encephalopathy, and Alzheimer disease, CA1 is the most vulnerable hippocampal region (18, 20–21). In contrast, as shown in this and previous reports (12, 13), the hippocampus of CJD brains most commonly harbors little pathology, and is better preserved than adjacent paleo- and neocortex.

Parvalbumin and calbindin-D28K are neuronal calcium-binding proteins that decorate distinct subsets of GABAergic neurons (10, 22). In the human hippocampus and EC, PV+ and Cal+ neurons were studied for their morphology and distribution (10, 11, 23), but have not been quantified so far. In controls, we observed a constant pattern of densities of PV+ neurons in distinct anatomical areas. The presence of PV+ neurons, variable involvement in pathology, and the fact that normal hippocampus expresses PrPc at higher levels than many other brain regions (24) make the ammon’s horn and adjacent areas useful for neuropathological correlation studies in prion diseases.

Loss of PV+ neurons was reported in stroke, HIV encephalitis, Alzheimer disease entorhinal cortex and CJD neocortex (9, 25–27). In contrast, PV+ neurons are well preserved in NMDA intoxication, Pick’s disease and Alzheimer disease neocortex (9, 28, 29). In transient cerebral ischemia, preservation of PV+ neurons (rat, gerbil) (30–32) or a slight transient decrease of PV immunoreactivity (rat) (33) was observed.

Some investigators (11) showed a preferential survival of Cal+ neurons and loss of PV+ neurons in cTLE hippocampi; thus, it might be argued that the changes we observed are secondary to mechanisms operating in cTLE. However, in our CJD or control hippocampi, any morphological abnormalities (neuronal loss and AG) characteristic for cTLE (19) were absent. Furthermore, loss of PV+ neurons in cTLE hippocampus was combined with severe neuronal loss of the region CA1-4, while loss of hippocampal PV+ neurons in CJD was most commonly (in 96% of the cases) the only pathological change in this region. Nevertheless, cTLE showed a preservation of PV+ neurons in Sub (11), PrS and TC, while in CJD PV+ neurons are lost in all investigated regions. This evidence suggests that widespread loss of PV+ neurons is a distinctive feature of CJD.

Although staining intensity (i.e. darkness) of PV labeling tends to decrease in autopsy cases, the general appearance of PV immunoreactivity is similar in autopsy cases and surgical specimens (11). Both our CJD and control material are from identically processed autopsy cases, and are therefore unlikely to label differently for technical reasons. There is also a theoretical possibility that loss of PV immunoreactivity in the CJD brain may reflect changed immunohistological characteristics of the protein (e.g.
Fig. 4. Calbindin immunostaining of a CJD (left) and a control (right) hippocampus. There was total loss of PV+ neurons in all investigated regions of the same CJD brain.

interaction with another protein, posttranslational modifications). However, this is unlikely in view of the fact that in almost all CJD cases a few otherwise normal PV+ neurons were left.

Typical symptoms in CJD (myoclonus, distinctive EEG pattern), neurophysiologic and morphologic changes in PrP knockout mice (6, 8), and other experimental evidence (4, 5, 7, 9, 34) suggest severe alteration of the GABAergic system in prion disease. We show that the normal density of PV+ neurons in control hippocampal regions topographically correlates with severity of tissue lesioning and that PV+ neurons are severely depleted or absent in CJD. Nevertheless, Cal+ neurons (another subset of GABAergic neurons) were mostly well preserved and reduced only in regions with extremely severe lesioning, thus seeming to be more resistant to damage in CJD. Loss of PV+ neurons might be an early event in the evolution of disease, followed later by spongiform change. This might explain the variable degree of spongiform change despite the widespread loss of PV+ neurons. In our data we support selective loss of PV+ inhibitory neurons as a possible pathogenetic basis for myoclonus and the distinctive EEG pattern of CJD (9).

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REFERENCES

11. Sloviter R, Sollas A, Barbano N, Laxer K. Calcium binding protein (calbindin D28K) and parvalbumin immunocytochemistry in the


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