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Research report

Reduced density of calbindin-immunoreactive interneurons in the planum temporale in schizophrenia

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Abstract

Reduced density of calbindin-containing interneurons in the prefrontal cortex in schizophrenia has been reported (Beasley et al 2002; Biol Psych 52:708–715). Calbindin is a calcium-binding protein (CBP) present in a subpopulation of GABAergic neurons restricted mainly to layer II of the cortex. A paraffin-embedded, 10-μm-thick section from the planum temporale (PT) of each hemisphere was prepared from 12 patients with schizophrenia and 12 controls. Calbindin-containing cells were stained using an antibody (D-28K). Counting frames were superimposed to sample within layer II of the PT. A bilateral reduction (20%) in calbindin cell density was found in patients (controlling for fixation time). Furthermore, mean calbindin cell cross-sectional area was increased in female patients and reduced in male patients. Reduced CBP expression (reducing the excitability of interneurons) or reduced number of CBP-containing cells may cause disinhibition of pyramidal cells. The majority of calbindin-containing cells in the mature brain are double-bouquet cells with vertically oriented dendrites and axon bundles. By exercising inhibitory modulation of pyramidal cells in a columnar arrangement, they make possible cohesive vertical inhibition of minicolumns. Loss of columnar inhibition may result in reduced minicolumnar segregation and altered cell size may reflect altered minicolumn size.

Theme: Disorders of the nervous system
Topic: Neuropsychiatric disorders

Keywords: Neurons; Calbindin; Schizophrenia; Minicolumn; Planum temporale cortex; Sex difference

1. Introduction

Calbindin is a calcium-binding protein (CBP) present in a subpopulation of GABAergic interneurons restricted mainly to layer II of the cerebral cortex. Reduced density of calbindin-containing interneurons in the prefrontal [2] and cingulate [11] cortex in schizophrenia has been reported. Reduced calbindin expression will reduce the excitability of interneurons causing disinhibition of pyramidal cells [18]. A reduction of the total number of calbindin-containing cells will have a similar disinhibitory effect.

Neurons containing other CBPs are not equally affected. Parvalbumin-containing interneurons are reduced in density in schizophrenia whereas calretinin-containing cells are not [18]. Calbindin-containing cells have a distinctive morphology. The majority of them in the mature brain are double-bouquet cells that are found mainly in layer II and have characteristic vertically oriented dendrites and axon bundles which form symmetrical synapses with dendritic shafts and spines of other cells [13]. Calbindin-immunoreactive double-bouquet axon bundles are spatially associated with myelinated axon bundles (originating from pyramidal cells) and have the same spacing as the vertical formations of cells described as minicolumns in human temporal neocortex [14].

Calbindin-containing cell density has not yet been reported for the superior temporal lobe in schizophrenia.

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The planum temporale (PT) is located in the superior temporal gyrus–a commonly altered structure in MRI studies–and is associated to the cortex linked to language lateralization [20], for which disturbed function [25] and asymmetry [19,28] have been reported in schizophrenia.

2. Materials and methods

2.1. Tissue

A collection of brain tissue from 12 normal comparison subjects and 12 patients with schizophrenia (conforming to DSM IV criteria) was used. Tissue was collected with consent in accordance with standard neuropathological practice and is registered with UK national investigations on organ retention. All cases are catalogued and none has been recalled. Cases were selected to yield comparable group mean fixation times and ages at death as far as possible, although group matching was not possible. Patients were included on the basis of the assessment of clinical notes by a consultant psychiatrist (T.J. Crow or Dr. S.J. Cooper, Belfast).

Pathological assessment of tissue samples was carried out by a consultant neuropathologist (M.M. Esiri or B McDonald, Oxford) and cases with significant pathology, such as Alzheimer's disease, were excluded using the CERAD criteria [27]. Demographic details and potentially confounding variables, including age at death, post-mortem interval, and fixation time, were subjected to statistical analysis (see Table 1).

2.2. Method

All tissue preparation and subsequent analyses were performed blind to diagnosis. Following removal of the leptomeninges, the temporal lobes were removed from the rest of the hemispheres at the posterior end of the Sylvian fissure. The planum temporale (PT) was identified on the superior surface of the temporal lobe, bounded anteriorly by Heschl's sulcus, posteriorly and laterally by the limits of the superior surface of the superior temporal gyrus (STG) in the Sylvian fissure, and painted with a green dye for easy identification on sections. A block of STG was removed 5 mm from the posterior Sylvian fissure along the antero-posterior axis and embedded in formalin. A coronal, 10-μm-thick section from the block of each hemisphere was cut on a rotary microtome, perpendicular to the long axis of the STG. Calbindin-containing cells were stained using an antibody (D-28K), and a Cresyl violet counter-stain for visualizing the background (see Fig. 1).

Table 1
Demographic details of the subject material (Mean and SD)

	Age at onset	Age at death	Post mortem interval	Time in formalin
Male comparison subjects (n = 5)	na	57.0 ± 13.2	42.2 ± 15.2	22.0 ± 9.9
Female comparison subjects (n = 7)	na	69.4 ± 13.2	33.7 ± 20.7	24.7 ± 11.6
Male schizophrenia subjects (n = 7)	26.4 ± 7.3	64.9 ± 14.4	37.6 ± 21.9	27.3 ± 19.1
Female schizophrenia subjects (n = 5)	32.0 ± 6.2	71.6 ± 17.6	29.6 ± 24.8	50.0 ± 23.3

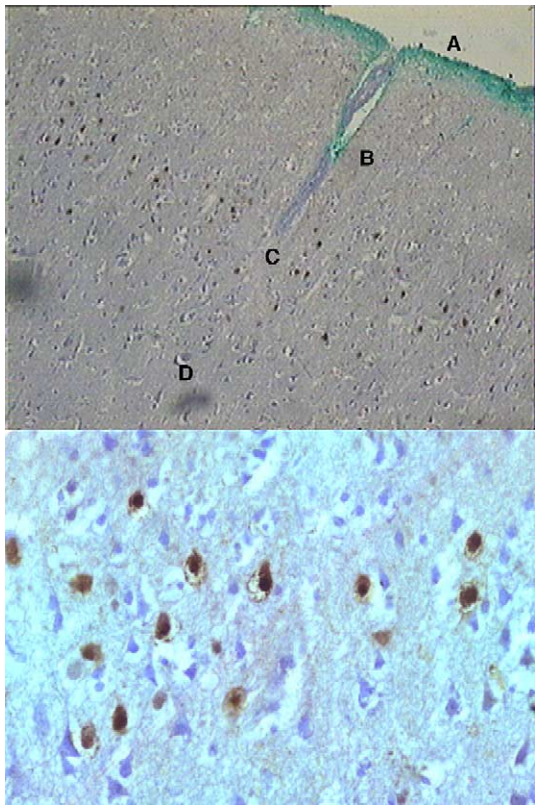

Fig. 1. (Above) Calbindin neurons are stained dark brown and mainly confined to lamina II. Other cells are blue (A = cortical surface (painted green), B = lamina I, C = lamina II, D = lamina III). A blood vessel can be seen penetrating the cortical surface down to lamina II. (Below) Higher magnification view of lamina II. A cluster of calbindin cells (brown) is interspersed with other cells (blue). Triangular-shaped blue-stained pyramidal neurons can be seen around the border with upper lamina III.

2.3. Immunohistochemistry

Sections were microwaved in antigen-unmasking solution (Vector H-3300) at low power (without boiling) for 30 min to improve antigen detection [17]. The tissue was pretreated with H_2O_2 and 10% host serum before a 2-day incubation at 4 °C with monoclonal antibody (mouse anti-Calbindin; D-28K from Swant, Switzerland) diluted 1:4000 in 0.1 M phosphate-buffered saline (PBS; pH 7.4). After a 30-min treatment with a 1:200 dilution of biotinylated goat anti-mouse-IgG (Sigma), the antibody was localized by the