The nucleus basalis (Ch4) in the alcoholic Wernicke-Korsakoff syndrome: reduced cell number in both amnesic and non-amnesic patients

K M Cullen, G M Halliday, D Caine, J J Kril

Abstract

Background—The cholinergic nucleus basalis (Ch4) is an exclusive site of neurofibrillary degeneration in alcoholic patients with Wernicke's encephalopathy.

Aim—To test the hypothesis that the loss of Ch4 neurons contributes to the memory disorder, Korsakoff's psychosis, commonly seen in Wernicke's encephalopathy.

Methods—Magnocellular basal forebrain neurons were quantified in alcoholic patients with Wernicke's encephalopathy, both with and without Korsakoff's psychosis, and neurologically asymptomatic alcoholic and non-alcoholic controls. Because amnesic and non-amnesic patients with Wernicke's encephalopathy share common periventricular lesions, both thiamine deficient groups as well as alcoholic patients with no neurological complications were included to determine the lesion specific to memory impairment.

Results—Ch4 cell number did not differ significantly between alcoholic and non-alcoholic controls and there was no correlation between cell number and lifetime alcohol intake. However, Ch4 cell number in all groups was significantly correlated with the volume of its major projection target, the cerebral cortex. Ch4 cell number in the non-amnesic Wernicke's encephalopathy group was significantly below controls (24%), with cell number in patients with Korsakoff's psychosis 21% below controls. There was considerable overlap in cell number between groups. On discriminant analysis, there was significantly greater cell loss in three non-amnesic patients with Wernicke's encephalopathy than in some patients with Korsakoff's psychosis. The non-amnesic patient with the greatest cell loss was impaired on attentional tasks.

Conclusion—Whereas neurons in the nucleus basalis are at risk in thiamine deficient alcoholic patients, cell loss is minor and does not account for the profound memory disorder.

Although there are several comprehensive descriptions of the neuropathology of Wernicke's encephalopathy, definitive anatomical substrates for the associated amnesic syndrome, Korsakoff's psychosis, have not been identified. We have previously shown that the cholinergic nucleus basalis (Ch4) is an exclusive site of neurofibrillary degeneration in thiamine deficient alcoholic patients. These findings prompted us to investigate the degree of Ch4 cell loss in association with the memory disorder Korsakoff's psychosis.

The notion that cholinergic deficits can result in memory dysfunction has been extensively evaluated in several animal and pharmacological models as well as in neurodegenerative diseases, most notably Alzheimer's disease. A role for the Ch4 in memory has been supported by a previous study of patients with Korsakoff's psychosis correlating loss of magnocellular basal forebrain neurons to memory dysfunction. To date this hypothesis remains largely unchallenged. The present study compared the Ch4 in patients with Wernicke's encephalopathy and those with additional Korsakoff's psychosis with age matched alcoholic and non-alcoholic controls to test the hypothesis that amnesic patients have a common anatomical lesion in the Ch4. As the Ch4 provides most of the cholinergic innervation to the cortex, cell number was also compared with the brain atrophy reported in alcoholic patients.

Materials and methods

CASE SELECTION

The present work is part of a broader investigation on memory specific lesions. All cases (table) were selected and classified retrospectively. The cases were evaluated as part of an assessment of operational criteria for the classification of chronic alcoholic patients without neurological abnormalities and alcoholic patients with Wernicke's encephalopathy or with Wernicke's encephalopathy and Korsakoff's psychosis. Written consent was obtained for hospital necropsies and the study was approved by the human ethics committee of the University of Sydney and Royal Prince Alfred Hospital under the New South Wales Transplantation and Anatomy Act. Details of diet and alcohol consumption were obtained from clinical records and written and telephone communication with general practition-
ers as well as from written questionnaires distributed to relatives of the patients. Most alcoholic patients (A1-A5, KP1, KP2, and KP4; table) were seen in the neuropsychology unit at Royal Prince Alfred Hospital; cases KP3 and WE4 were seen at the Royal Brisbane Hospital.

Strict exclusion criteria were employed for the purposes of this study: cases were not included if there was evidence of stroke, head injury, or Alzheimer type cortical pathology (neuritic plaques and neurofibrillary tangles), if details of alcohol and thiamine status could not be ascertained, or if time from death to postmortem was greater than 72 hours. All cases of Wernicke's encephalopathy studied, including those with Korsakoff's psychosis, had documentation in medical records of at least two of the classic triad of symptoms, although most had evidence of all three. Our previous work has shown that neurological evidence of Wernicke's encephalopathy was present in all alcoholic cases excluding case WE4 (brain-stem) and KP4 (hypothalamus). All other cases of Wernicke's encephalopathy had persisting neurological signs and were classified as chronic. In addition to the classic signs of Wernicke's encephalopathy, cases WE2 and WE3 also showed frontal lobe signs (including abnormalities in planning, insight, or abstraction), cases WE3 and WE5 had mild intermittent memory dysfunction (defined as inability to remember at least two words in a four item test or mild impairment on more elaborate neuropsychological tests).

### Patient details

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<th>Case</th>
<th>Age (y)</th>
<th>Sex (M/F)</th>
<th>Delay pm (h)</th>
<th>Duration of disease</th>
<th>Ch4 cell number</th>
<th>Ch4 mean cell diameter (μm)</th>
<th>Volume cerebral hemispheres (cm³)</th>
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stable and persistent inability to form new memories. Case KP2 also had frontal lobe signs.

**Tissue Preparation**
The brains were removed at necropsy and fixed by immersion in 15% formalin (pH 7.4) for at least two weeks. Whole brain and cerebrum weights and volumes (by fluid displacement) were determined before and after fixation to assess shrinkage artefact (< 5% in each case). The cerebrum was embedded in agar and sliced coronally on a rotary slicer at 3 mm intervals for macroscopic diagnosis and photographed at 1× magnification for volume determination. Cortical, brainstem, diencephalic, and cerebellar blocks were taken for routine histopathological evaluation. The basal forebrain from both hemispheres was removed and the blocks cryoprotected in Tris-HCl buffer pH 7.4 containing 30% sucrose for two to three days, before serial sectioning at 50 μm on a Leitz freezing microtome. Six series (1/15) of sections were stained with haematoxylin and eosis, a modified Bielschowsky silver stain, luxol fast blue, cresyl violet, and immunohistochemistry for tau and the calcium binding protein, calbindin-D 28k. Calbindin has a high specificity for colocalisation with cholinergic specific markers, supporting its role as a specific marker for the Ch4 in the basal forebrain. For immunohistochemistry, calbindin-D 28k (1:200, Sigma, C8666) and tau (1:10 000, Sigma, T5530) binding were visualised using appropriate biotinylated secondary antibodies and the avidin-biotin peroxidase complex detection system (ABC kit, Vector Laboratories, Burlingame, CA, USA) with dianinobenzidine as the chromogen, described in detail previously.

**Analysis**
Our previous cytoarchitectural description of the human basal forebrain was used to delineate subregions of the Ch4: anteromedial, anterolateral, dorsal and ventral intermediate, and posterior. The volume of the Ch4, neuronal number, and mean diameter were determined in a complete series of Nissl stained sections with the aid of an NEC computer and Magellan software. Computer reconstructions of the Ch4 nucleus were created for each case to assess the distribution of cell loss. The volumes of the cerebral hemispheres, cortical grey, subcortical grey, and white matter (subcortical white matter, internal and external capsules, corpus callosum and anterior commissure) were determined by point counting on complete series of black and white photographs of 3 mm brain slices, as previously described. The volumes of the cerebral hemispheres, cortical grey matter, and white matter were then compared with Ch4 cell number using regression analysis.

Quantitative results were derived with observers blind to the clinical diagnosis of the case, including memory status. Data from all brain regions were acquired independently. Patient differences were assessed using analysis of variance (ANOVA) with protected t tests. Discriminant analysis was used to determine deviations of individual cases from diagnostic groupings on the basis of Ch4 cell number and cortical volumes. Relations between patient variables and Ch4 cell number were tested using regression analysis. Quantitative results from two observers were not significantly different in t tests (P>0.05). No Ch4 variable was significantly correlated to patients’ post-mortem delay, sex, or cause of death.

**Results**
In the vicinity of the Ch4, gross morphological changes including forebrain atrophy, ventricular dilatation, and glial scarring disrupted the normal forebrain architecture and gave an irregular concave appearance to the base of the brain in all cases of Wernicke’s encephalopathy. In these cases, the Ch4 nucleus appeared as a narrower, denser band of cells along the basal surface of the brain compared with alcoholic patients without Wernicke’s encephalopathy and controls. Accumulation of lipofuscin pigment was pronounced in all alcoholic patients, particularly cases WE1, WE2, WE5, and KP3.

In all cases, alcoholic and non-alcoholic, most Nissl stained magnocellular neurons were calcinbind immunoreactive (95%, P<0.05). Reduced numbers of calbindin positive fibres within Ch4 cell groups was seen in several cases of Wernicke’s encephalopathy, most notably WE4 and WE5, and cases of Korsakoff’s psychosis, KP3 and KP4. Comparison of cell size of Ch4 neurons disclosed no difference in the mean cell diameters between Korsakoff’s psychosis, Wernicke’s encephalopathy, alcoholic, and non-alcoholic control groups (table), although mean cell diameters in cases WE5 and KP4 were significantly below group means.

There was no significant difference in cell number between alcoholic controls and non-alcoholic age matched controls (P>0.05, ANOVA; figure, A). The mean number of Ch4 neurons in the Korsakoff’s psychosis and Wernicke’s encephalopathy groups differed significantly from non-alcoholic control groups (P<0.02, Fisher’s protected t test; figure, A), with the Wernicke’s encephalopathy mean 24% and the Korsakoff’s psychosis mean 21% below that of controls. The Wernicke’s encephalopathy group was significantly below alcoholic controls (21%, P<0.02), but the difference did not reach significance for the Korsakoff’s psychosis group (18%, P>0.06). There was substantial overlap between all groups. Discriminant analysis showed that on the basis of age and Ch4 cell number, cases WE1 and KP1 were within control group ranges (F2,9=4.6, P=0.026) and the alcoholic and non-alcoholic groups could not be distinguished (F2,9=0.8, P=0.47).

A homogeneous reduction of cell number per 50 μm section was noted in all subregions of the Ch4 indicating that no focal loss of neurons occurred in any patients with Wernicke’s encephalopathy or Korsakoff’s psychosis. Despite low neuronal counts in some patients with Wernicke’s encephalopathy and Korsakoff’s psychosis compared with controls, no consistent decrease in packing density was
Changes, particularly case A5. Group, with gliosis accompanying density noted in most cases in the non-WE alcoholic KP4. Increased density was the only change cases of Wernicke's encephalopathy, WE5 and most alcoholic cases, with the exception of noted. An increased cell density was seen in alcoholic and non-alcoholic control groups, both thiamine-deficient groups did significantly differ from controls on discriminant analysis (F_{2,17}=4.6, P=0.026).

Measurement of cerebral volumes in controls and alcoholic groups disclosed atrophy of white matter but not cortical grey matter with high alcohol intake (table). Both cerebral hemisphere and cortical grey matter volumes were significantly correlated with Ch4 cell number in alcoholic and non-alcoholic control groups, and this correlation was preserved in the Wernicke's encephalopathy and Korsakoff's psychosis groups (r^2=0.385, P<0.002; all cases together; figure, C).

Discussion

The present study examined Ch4 cell number and size in controls, alcoholic patients without neurological complications, and alcoholic patients with Wernicke's encephalopathy. Our aim was to test the hypotheses that (1) Ch4 cell loss distinguishes the amnesic from the non-amnesic patients with Wernicke's encephalopathy and (2) Ch4 cell loss correlates with alcohol intake. Our results corroborate earlier reports that cell number remains unchanged in alcoholic patients without Wernicke's encephalopathy. The data also show that Ch4 cell number is reduced in patients with Wernicke's encephalopathy both with and without Korsakoff's psychosis, consistent with our previous finding of neurofibrillary tangles in the Ch4 of alcoholic patients with Wernicke's encephalopathy. Evidence of cell loss in non-amnesic patients with Wernicke's encephalopathy, however, refutes previous, yet unchallenged, conclusions of a causal relation between reduced Ch4 cell number and memory loss. These results cast doubt on the notion that alcoholic Korsakoff's psychosis is a "basal forebrain" amnesia.

Despite the continued popularity of the cholinergic hypothesis, interpretation of the Ch4 deficit in Korsakoff's psychosis has been confounded by several factors, including controversial clinical definitions of the amnesia. Recently devised operational criteria strictly define Korsakoff's psychosis as a stable amnesic syndrome occurring in patients with Wernicke's encephalopathy against a background of an intact sensorium. Using this definition, patients with Wernicke's encephalopathy with and without Korsakoff's psychosis can be classified with a high degree of certainty. Whereas several of the patients with Wernicke's encephalopathy in the present study exhibited acute confusion and disorientation and mild memory impairment, memory functions were restored when the Wernicke's encephalopathy episode cleared. Thus two groups, both of which showed the characteristic neuropathological lesions of Wernicke's encephalopathy, could be distinguished clinically: (1) patients with Wernicke's encephalopathy with classic neurological signs and (2) patients with Wernicke's encephalopathy with neurological signs accompanied by a stable,
The nucleus basalis (Ch4) in the Wernicke-Korsako syndrome

persistent amnesia. By contrast, previous studies of the Ch4 have not included non-amnesic patients with Wernicke's encephalopathy, despite documentation that Korsakoff's psychosis is not an inevitable outcome of Wernicke's encephalopathy. Earlier studies also included an alcoholic patient group with Korsakoff's psychosis but without Wernicke's encephalopathy that could not be distinguished in our necropsy series of more than 4000 cases. We suggest that if current operational criteria were employed, all such alcoholic patients with Korsakoff's psychosis would show evidence of Wernicke's encephalopathy pathology.

Although Ch4 cell loss cannot account for the amnesia of Korsakoff's psychosis, lower Ch4 cell number may contribute to cognitive dysfunction in patients with Wernicke's encephalopathy, both with and without Korsakoff's psychosis. In the present study, alcoholic patients with Wernicke's encephalopathy but without Korsakoff's psychosis exhibited mild cognitive impairment, including intermittent disorientation and frontal lobe signs such as impaired planning and abstraction. Memory difficulties, such as reduced digit span, were noted in three cases and case WE5 showed specific deficits in attention and difficulty in the inhibition of irrelevant information. This last case (showing the greatest Ch4 cell loss) provides support for a correlation between cholinergic lesions and pronounced attentional deficits but not memory loss itself, as has been postulated from animal models. Thus Ch4 cell loss may be relevant to the clinical picture of Korsakoff's psychosis, which includes deficits in sustained attention and sensitivity to interference in addition to the striking anterograde amnesia.

The mild Ch4 cell loss in chronic Wernicke's encephalopathy (with or without Korsakoff's psychosis), reduced cell size in only two cases (WE5 and KP4), with the most extensive cell loss and the absence of cell loss in acute Wernicke's encephalopathy is in stark contrast to the gross necrotic lesions in the hypothalamus and thalamus of these patients. Several scenarios may account for the difference in severity between basal forebrain and diencephalic pathology. As the Ch4 has a substantial projection to the mediodorsal nucleus of the thalamus, a key site of pathology in Wernicke's encephalopathy, cell loss may occur via a retrograde mechanism rather than via direct insult.

The correlation between cortical atrophy and Ch4 cell number may also reflect an anterograde or retrograde degeneration. Primary changes to parenchymal arterioles, consisting of endothelial swelling, dilatation, smooth muscle and elastin degeneration, and petechial haemorrhages have been noted in thiamine deficiency. The cholinergic forebrain nuclei projections have been shown to contact parenchymal blood vessels, with a putative role in regulation of local cerebral blood flow. Damage to the thalamic or cortical vasculature as a primary degenerative mechanism in Wernicke's encephalopathy may result in secondary loss of Ch4 cells via neurofibrillary tangle formation. In this light, it is interesting to note that neurofibrillary tangles in Ch4 have been noted ipsilaterally to a cerebral infarct.

There has been considerable debate as to the relative contributions of ethanol intake and nutritional deficiency to alcoholic brain damage. Most examples of alcoholic dementia may be attributable to metabolic or nutritional complications of alcoholism; however, thiamine deficiency must apparently occur on a background of high alcohol intake for development of the full Wernicke-Korsakoff syndrome. Despite histories of high alcohol intake (both amount consumed per day and lifetime intake) in all alcoholic patients studied, Loss of Ch4 cells occurred only in those with evidence of long term dietary deficiency and no loss was seen after a single acute episode of Wernicke's encephalopathy. This corroborates our previous work showing neurofibrillary tangle formation only in alcoholic patients with chronic Wernicke's encephalopathy and further supports a relation between thiamine deficiency, but not alcohol intake alone, and Ch4 cell loss. By contrast, rat models of chronic ethanol intoxication show a correlation of memory dysfunction with basal forebrain cell loss or reduced cholinergic input to the hippocampus, with cholinergic rich transplants improving performance on behavioural tests. These studies suggest that, at least in rodents, alcohol is a primary neurotoxin that can cause memory dysfunction. To reconcile human studies with those in rats, it could be conjectured that the Ch4 homologue in rats is more vulnerable to alcohol toxicity than is the human Ch4. Indeed, Ch4 neurochemistry differs between rodents, non-human primates, and humans. However, as ethanol reduces thiamine absorption, malnutrition may be an unrecognised confounder in studies of alcoholic fed rats. Consistent with this notion, experimentally induced thiamine deficiency can produce a cholinergic deficit in rats.

Conclusion

Although cholinergic involvement in the amnesic syndrome is widely cited in the literature on Korsakoff's psychosis, the studies supporting the hypothesis have lacked appropriate controls. Our results corroborate previous work showing Ch4 cell loss in Korsakoff's psychosis; however, because a similar lesion was also seen in non-amnesic Wernicke's encephalopathy, we cannot conclude that Ch4 loss constitutes the definitive anatomical substrate for the amnesia. These findings contribute important information that considerably alters the general conclusions of previous postmortem studies of alcoholic Korsakoff's psychosis. Nevertheless, if it is assumed that Ch4 pathology is not silent in alcoholic patients with Wernicke's encephalopathy, a component of their cognitive impairment, such as attentional deficits, may be attributable to cholinergic cell loss. Our results did not support a critical role for the Ch4 in memory loss in Korsakoff's psychosis, but provide further support for a more circumscribed role for the Ch4 in attention. This
finding has direct clinical relevance as treatment of patients with Korsakoff’s psychosis with cholinergic replacement therapy (tetrahydroaminoacridine) has been suggested.13

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