Degeneration of anterior thalamic nuclei differentiates alcoholics with amnesia

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Summary
The specific neural substrate underlying the amnesia in alcoholic Korsakoff’s psychosis is poorly defined because of the considerable brain damage found in many non-amnesic alcoholics, particularly those with Wernicke’s encephalopathy. Using operational criteria to identify alcoholics with and without Korsakoff’s psychosis, we have shown that many of the cortical and subcortical regions involved in the encoding and retrieval of episodic memory are either unaffected (hippocampus) or damaged to the same extent (prefrontal cortex and the cholinergic basal forebrain) in both amnesic and non-amnesic alcoholics. In the present study we analysed the diencephalic regions involved in episodic memory to determine the neural substrate for the amnesia observed in alcoholic Korsakoff’s psychosis. The number of neurons in spaced serial sections containing the hypothalamic mamillary nuclei and the anterior and mediodorsal thalamic nuclei was estimated using unbiased stereological techniques. Neurodegeneration of the hypothalamic mamillary nuclei and the mediodorsal thalamic nuclei was substantial in both non-amnesic and amnesic alcoholics with Wernicke’s encephalopathy. However, neuronal loss in the anterior thalamic nuclei was found consistently only in alcoholic Korsakoff’s psychosis. This is the first demonstration of a differentiating lesion in alcoholic Korsakoff’s psychosis and supports previous evidence that degeneration of thalamic relays are important in this memory disorder.

Keywords: alcohol-related brain damage; Korsakoff’s psychosis; mamillary body; neurodegeneration; thalamus

Introduction
Analysis of patients with well-characterized brain lesions has revealed that many neural circuits are involved in the long-term representation of memory (Squire et al., 1993; Zola-Morgan and Squire, 1993; Markowitsch, 1995; Mayes, 1995; Kopelman, 1995; Aggleton and Shaw, 1996; Gordon, 1997; Kroll et al., 1997; Poldrack and Gabrieli, 1997; Weiskrantz, 1997; Gabrieli, 1998; Sziklas and Petrides, 1998). In particular, such studies have highlighted the role of medial temporal lobe circuits in conscious memory for facts and events (declarative memory) and suggest further organizational differences between episodic memory (the conscious remembering of individual events occupying particular spatial and temporal contexts) and semantic memory (the organized knowledge a person has about the world). One of the largest patient groups used in such studies are those with alcoholic Korsakoff’s psychosis. The clinical diagnosis of Korsakoff’s psychosis in alcoholics has only recently been standardized (Caine et al., 1997) with the amnesic syndrome characterized by persistent anterograde episodic memory loss and preserved semantic memory, intelligence and learned behaviour (Kopelman, 1995). Korsakoff’s psychosis occurs most commonly in nutritionally compromised alcoholics and, until recently, was thought to be the inevitable sequela of an acute episode of Wernicke’s encephalopathy caused by thiamine deficiency (Ryan and Butters, 1980). However, we have recently shown in a large population study that Korsakoff’s psychosis is not inevitable in alcoholics with Wernicke’s encephalopathy (Caine et al., 1997). All alcoholics with Wernicke’s encephalopathy have characteristic diencephalic lesions (Torvik, 1987; Victor et al., 1989) but only some have Korsakoff’s psychosis.

The location of the lesion(s) responsible for the amnesia in Korsakoff’s psychosis is not known. We have addressed this issue by performing a number of detailed neuropathological studies. Neuroanatomical regions examined include the hippocampus (Harding et al., 1997) and cerebral cortex (Kril et al., 1997).
et al., 1997) as well as several subcortical regions thought to significantly influence cortical activity [brainstem serotonergic nuclei (Halliday et al., 1993), the noradrenergic locus coeruleus (Halliday et al., 1992), the cholinergic basal forebrain (Cullen et al., 1997) and vasopressin neurons in the hypothalamus ( Harding et al., 1996)]. With the exception of the locus coeruleus and hippocampus, quantification revealed significant neuronal degeneration in all of these non-thalamic regions with the degree of degeneration similar in alcoholics with and without Korsakoff’s psychosis. This suggests that damage to these sites is not sufficient to cause a persisting amnesic syndrome. Previous studies of the diencephalon in similar cases concentrated on lesion site rather than the degree of neuronal damage. The aim of the present study is to quantify diencephalic neuronal loss in well-studied alcoholics with and without Korsakoff’s psychosis in thalamic regions previously identified as having significant neuropathology and known to be important for the retrieval of episodic memory.

Material and methods

Autopsy material

Brains were obtained from a large autopsy series (4600 patients) from the Institute of Forensic Medicine (Sydney) and Royal Prince Alfred Hospital, as has been previously described in detail (Caine et al., 1997). Consent was obtained from the next of kin for hospital autopsies. Post-mortem delay was <42 h (mean 16 ± 9 h) in all cases. The study was approved by the ethics committees of the University of Sydney and the Central Sydney Area Health Service. A full necropsy was performed for all patients, including macroscopic and microscopic examination of all organs, and a detailed, standardized neuropathological examination as previously described (Caine et al., 1997). Briefly, for neuropathological examination the brains were weighed following removal from the cranium and fixed by suspension in 15% formalin for 2 weeks. Following fixation, brains were re-weighed and the volume determined by water displacement. The cerebrum was separated from the cerebellum and brainstem by sectioning at the level of the superior colliculus. The cerebrum was embedded in agar, sectioned at 3 mm intervals in the coronal plane and standardized blocks of tissue taken and stained for extensive and systematic neuropathological examination and classification. Pathological Wernicke’s encephalopathy was diagnosed when mamillary body and periventricular lesions were evident (Torvik, 1987; Victor et al., 1989; Kril, 1996). In acute Wernicke’s encephalopathy the pathology was largely confined to abnormalities of blood vessels with petechial haemorrhages common. In chronic Wernicke’s encephalopathy neuronal loss and gliosis were evident in the mamillary bodies. A diagnosis of normal brain was made if no neuropathological abnormality was detected. Cases with other neuropathological abnormalities were excluded.

A range of clinical signs and symptoms representing deficits in the major clinical domains known to be affected in alcoholics was evaluated using standardized operational criteria (Caine et al., 1997). Briefly, the 11 commonly encountered clinical domains identified were assessed: Wernicke’s encephalopathy (diagnosed during life), dietary deficiencies (a body mass index lower than 2 SD below normal as evidence of undernutrition, a history of grossly impaired dietary intake or an abnormal thiamine status), eye signs (oculomotor abnormalities such as ophthalmoplegia, nystagmus or gaze palsy), cerebellar signs (ataxia, unsteadiness, abnormalities of past pointing, dysdiadochokinesia or impaired heel–shin testing), seizures (either as part of a withdrawal syndrome or in isolation, or a long-standing history of anticonvulsant medication), frontal lobe dysfunction (abnormalities in planning, insight or abstraction with formal neuropsychological testing or when neurological examination elicited these characteristics), amnesia (a stable and persisting inability to form new memories), mild memory impairment (failure to remember two or more words in the four-item memory test, or impairment on more elaborate neuropsychological tests of memory function), altered mental state (disorientation in two of three fields, confused, an abnormal digit span or comatose), hepatic encephalopathy (grades 3 or 4, in which signs of impaired mental state range from confusion to coma) and liver disease (signs of systematic abnormalities secondary to chronic liver disease including malaena, haematemesis, jaundice, ascites or asterixis).

Clinicopathological criteria for case selection and classification

Cases were carefully selected using operational criteria (Caine et al., 1997) proven to accurately classify patients based on their neuropathology, alcohol consumption histories and the presence or absence of characteristic clinical signs and symptoms. Seven per cent of the autopsy series of 4600 patients had a history of alcoholism based on information from telephone interviews to general practitioners, written questionnaires to relatives and clinical details from hospital records. Alcohol consumption was calculated in grams of absolute ethanol per day, as previously described ( Harding et al., 1996). A mean of the maximum and minimum rates was used for classification (alcoholics consumed >80 g of alcohol per day for the majority of their adult lives), as recorded from the multiple sources described above. Lifetime alcohol consumption (mean daily intake × 365 × duration of consumption in years) was also calculated.

Exclusion criteria were an average daily alcohol consumption of 20–80 g, inadequate clinical history (no neurological examination or single hospital admission only) or neuropathological evidence of a cerebral infarction, head injury or neurodegenerative disease (e.g. Alzheimer’s disease). For the present study, cases with the following clinical and/or pathological signs were also excluded from
further analysis: seizures, hepatic encephalopathy and liver disease. Pathologically, cases with severe liver disease had liver cirrhosis or hepatitis, and cases with hepatic encephalopathy had liver disease and Alzheimer type II astrocytes in the brain (common in basal ganglia, cerebral cortex or pons).

Of the original sample, only eight alcoholics had amnesia without significant liver disease. All cases were selected for analysis. These amnesic cases were all institutionalized with long documented histories of their permanent memory impairment (2–20 years). Formal clinical and neuropsychological testing was performed in all cases and all met current criteria for Korsakoff’s psychosis (Butters and Stuss, 1991; Kopelman, 1995; O’Connor et al., 1995). In all but one case both serial test scores and documented clinical observation revealed a stable and persisting inability to form new memories. In one case adequate neuropsychological testing occurred on only one occasion just prior to death in 1991, but there was ample clinical evidence of a persistent memory deficit dating back to 1982. All amnesic cases had chronic Wernicke’s encephalopathy pathology with typical mamillary body and periventricular neuronal loss and gliosis (Torvik, 1987; Victor et al., 1989; Kril, 1996).

For comparison, five non-amnesic alcoholics with Wernicke’s encephalopathy and without other neurological pathology, as well as five non-amnesic alcoholics without neuropathology or neurological complications, were selected. These cases were selected because they all had full clinical and neuropsychological assessments close to the time of death (2 weeks to 6 months prior to death). Patients underwent a 90 min clinical assessment by a consultant neurologist or neurology registrar, comprising a detailed history following a standard protocol, a complete general medical examination and a detailed neurological examination including an examination of mental status. They also underwent clinical neuropsychological assessment which included standardized administration and scoring of the following neuropsychological tests: Wechsler Memory Scale, Rey Auditory Verbal Learning Test, Rey–Osterreith Complex Figures Test, Trail Making Test and Stroop Test (Spreen and Strauss, 1991). Repeated examination of non-amnesic alcoholics with Wernicke’s encephalopathy revealed fluctuations in their cognitive status such that at some time after the diagnosis of Wernicke’s encephalopathy they were alert, oriented and capable of learning at least two of four items on mental status examination, or of retaining some of the words from the Rey Auditory Verbal Learning Test after administration of the interference trial. Pathological Wernicke’s encephalopathy was diagnosed in all cases (mamillary body and periventricular lesions; Torvik, 1987; Victor et al., 1989; Kril, 1996). Non-amnesic alcoholics without Wernicke’s encephalopathy met the same criteria on memory testing and had no neuropathological abnormalities.

In addition to these alcoholic cases, six age- and sex-matched non-alcoholic controls were chosen from the sample using the operational criteria. All non-alcoholic controls consumed <20 g of alcohol per day (most drank no alcohol) and had no neuropathological abnormality. Details of the age, sex, clinicopathological classification, and the clinical signs and symptoms for each case selected for analysis are presented in Table 1.

**Tissue preparation and analysis**

The left and right diencephalon were dissected from brain slices from the decussation of the anterior commissure through to the posterior limit of the pulvinar nucleus. Diencephalic blocks were cryoprotected in 30% sucrose in 0.1 M Tris–HCl buffer at 4°C for 48–60 h, serially sectioned at 50 µm on a freezing microtome (Leitz Instruments) and every 15th serial section stained with buffered cresyl violet (0.5% in acetate buffer, pH 5.3) for neuronal cell counts. The accuracy of the instrument to cut 50 µm sections has been previously verified (Harding et al., 1994). Subsequent serial sections were stained with haematoxylin and eosin for cell pathology, luxol fast blue for myelin structure and the modified Bielschowsky silver stain for fibre pathology. The boundaries of the various thalamic nuclei were defined by their cytoarchitecture using the atlas of the thalamus published by Hirai and Jones (Hirai and Jones, 1989).

The total volume of the thalamus was calculated by point-counting enlarged photographic images of the thalamus from the 3 mm coronal brain slices (co-efficient of error = 0.03) and applying Cavalieri’s principle of volume estimation. To analyse smaller regions, the use of a high quality binocular microscope with a rotatable stage and a camera lucida attachment was necessary. This allowed the superimposition of a computer image with the optical image of the microscope for data input. A computer mouse allowed the operator to accurately trace the outline of the structures under examination. Separate tracings were made of the medial mamillary nucleus (MM), mediodorsal nucleus (MD), anterior principal nucleus (AP) and pulvinar, and the areas calculated for each structure in sections spaced every 750 µm (co-efficient of error = 0.13, 0.06, 0.05 and 0.03, respectively). The total volume for each nucleus was calculated by applying Cavalieri’s principle of volume estimation. The volume of the remaining ventral and dorsal thalamic tiers (lateral groups) was calculated by subtracting the volume of the midline regions measured from the total thalamic volume.

The unbiased optical dissector technique was used to systematically quantify neurons in the diencephalic limbic relay nuclei. Our preliminary studies (Harding et al., 1994) revealed that the most efficient dissector area for neurons in 50 µm sections using our microscope set-up was 140 × 140 µm, placed 1 mm (AP and MM) or 2 mm (MD) apart. The number of sections upon which dissector frames were placed varied from 12 to 38 between cases, with the number of dissector frames also varying between cases (range 21–165). Neurons whose nucleolus fell entirely within the sampling frame or on one of two adjacent inclusion borders were counted. The number of neurons counted varied between 58...
analyses. There were no correlations between any (P > 0.05). This indicates no systematic laterality and therefore
Left/right differences were tested using paired t tests and were not significant for any variable in any group (P > 0.05). This indicates no systematic laterality and therefore

and 538 for the MM, 43 and 597 for the MD, and 113 and 461 for the AP. Repeated measures of the number of neurons within the sampling frames in multiple sections from multiple cases always gave similar results, even between different investigators. The density of neurons was determined by dividing the total number of neurons counted by the total sample volume. We have previously demonstrated that the use of the full section thickness does not significantly alter neuronal density measurement (Harding et al., 1994). The total number of neurons was estimated by multiplying the density of the neurons (co-efficient of error range = 0.05–0.11) by the volume in which they were contained.

**Data analysis**

Tissue quantification was performed without knowledge of case classification, although the pathology of Wernicke’s encephalopathy was obvious during the quantification. Statistical analysis was performed on Statview 4.0 program (Abacus Concepts, Berkeley, Calif., USA). Means ± standard deviations are given for all variables and a P value <0.05 accepted as statistically significant. There was no correlation between any measure and post-mortem delay or brain weight. Left/right differences were tested using paired t tests and were not significant for any variable in any group (P > 0.05). This indicates no systematic laterality and therefore the left and right measures were summed for all further analyses. There were no correlations between any diencephalic measures and age, alcohol intake (maximum daily rate and lifetime amount) or duration of alcohol consumption using linear regression analyses. Single factor ANOVA (analysis of variance) for neuronal number and volumes with diagnostic group as the nominal variable were performed and, if significant, the mean group values were further tested using post hoc Fishers’ protected least square difference test.

**Results**

**Clinicopathological variables**

There were no significant differences in the age, brain weight or brain volume between any of the diagnostic groups. As expected, there were significant differences between the groups in the amount of alcohol consumed because controls consumed almost no alcohol compared with all chronic alcoholics consuming over 80 g of alcohol per day \[F(3,20) = 12, \ P < 0.0001, \ post hoc P \] values for controls versus Korsakoff’s psychosis \(P = 0.0006, \) for controls versus Wernicke’s encephalopathy alone \(P < 0.0001, \) for non-alcoholic controls versus alcoholic controls \(P = 0.002, \) for alcoholic controls versus Wernicke’s encephalopathy \(P = 0.17, \) for alcoholic controls versus Korsakoff’s psychosis \(P = 0.96, \) for Wernicke’s encephalopathy versus Korsakoff’s psychosis \(P = 0.14. \) There was no significant difference \((P > 0.1)\) in the amount of alcohol consumed between

### Table 1 Case details of subjects including clinicopathological diagnosis and duration of alcoholism

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<th>Diagnosis</th>
<th>Age (years)</th>
<th>Sex</th>
<th>Duration (years)</th>
<th>Clinical WE</th>
<th>Eye signs</th>
<th>Cerebellar signs</th>
<th>Amnesia</th>
<th>Mild memory loss</th>
<th>Altered mental state</th>
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KP = Korsakoff’s psychosis; WE = Wernicke’s encephalopathy (all had dietary deficiencies); M = male; F = female; + = clinical sign present.
any of the chronic alcoholics with or without Wernicke’s encephalopathy or Korsakoff’s psychosis (maximum daily alcohol consumption of 230 ± 90 g for 32 ± 7 years with an average lifetime consumption of 2300 ± 1600 kg).

While the duration of Wernicke’s encephalopathy was not significantly different between those with Wernicke’s encephalopathy alone versus those with additional Korsakoff’s psychosis (Wernicke’s encephalopathy duration...
4 ± 5 years, Wernicke’s encephalopathy in amnesic cases (8 ± 5 years), three of the five alcoholics with Wernicke’s encephalopathy alone had durations of <12 months, whereas none of the eight alcoholics with Korsakoff’s psychosis had similarly short durations of Wernicke’s encephalopathy.

### Diencephalic damage

As previously described (Torkk, 1987; Victor et al., 1989; Kril, 1996), only alcoholics with Wernicke’s encephalopathy (n = 13) had diencephalic damage with vascular hyperplasia and hypertrophy common in the midline regions of the thalamus and hypothalamus.

Symmetric petechial microhaemorrhages and central necrosis in the mamillary bodies with early vascular and endothelial proliferation in periventricular regions (Fig. 1D) were seen in 8 out of 13 cases, whereas evidence of chronic Wernicke’s encephalopathy, namely discoloured and shrunken mamillary bodies (Fig. 1A and B), gliosis, spongiosis and patchy neuronal loss, was seen in 12 out of 13 cases. Neuronal pathology including neuronophagia (Fig. 1C), swelling, satellitosis and loss of Nissl substance was common. The volume of the MM was reduced in the amnesic cases compared with the other groups [F(3, 20) = 4.9, P = 0.01; post hoc P values for controls versus Korsakoff’s psychosis P = 0.002, for controls versus Wernicke’s encephalopathy alone P = 0.28, for non-alcoholic controls versus alcoholic controls P = 0.64, for alcoholic controls versus Wernicke’s encephalopathy P = 0.54, for alcoholic controls versus Korsakoff’s psychosis P = 0.01, for Wernicke’s encephalopathy versus Korsakoff’s psychosis P = 0.047], although there was considerable variability in this measure in the alcoholics with Wernicke’s encephalopathy alone (Table 2). There was no correlation between tissue volume loss in the MM and cerebrum (r^2 = 0.006, P = 0.30). However, MM tissue atrophy was correlated with the duration of Wernicke’s encephalopathy, accounting for much of the variability in this measure (r^2 = 0.62, P = 0.002; Fig. 2A). Significant volume changes were only found in alcoholics with durations of Wernicke’s encephalopathy of greater than 12 months (Fig. 2A). Thus, although all alcoholics with Wernicke’s encephalopathy by definition had significant vascular pathology and neuronal loss in the MM, atrophy of this region appears to occur only after the first episode of clinical Wernicke’s encephalopathy. The cell loss in the hypothalamus appeared restricted to the MM.

There was neuropathology in the medial thalamus in all alcoholics with Wernicke’s encephalopathy, with the total thalamic volume significantly smaller in these alcoholics [F(3, 20) = 6.4, P = 0.003; post hoc P values for controls versus Korsakoff’s psychosis P = 0.0005, for controls versus Wernicke’s encephalopathy alone P = 0.009, for non-alcoholic controls versus alcoholic controls P = 0.18, for alcoholic controls versus Wernicke’s encephalopathy P = 0.17, for alcoholic controls versus Korsakoff’s psychosis P = 0.02, for Wernicke’s encephalopathy versus Korsakoff’s psychosis P = 0.40; Fig. 2B]. In contrast to the MM, thalamic atrophy correlated with atrophy of the cerebrum (r^2 = 0.61, P < 0.0001; Fig. 2C). Of all thalamic regions, the MD was most consistently affected by the neuropathology of Wernicke’s encephalopathy. In all alcoholics with Wernicke’s encephalopathy there was considerable vascular hypertrophy and hyperplasia with variable neuronal loss (Figs 3 and 4). The combined volume of the MD and AP was significantly reduced in alcoholics with Korsakoff’s psychosis compared with alcoholic and non-alcoholic controls or alcoholics with Wernicke’s encephalopathy alone [F(3, 20) = 9.2, P = 0.0005; post hoc P values for controls versus Korsakoff’s psychosis P < 0.0001, for controls versus Wernicke’s encephalopathy alone P = 0.11, for non-alcoholic controls versus alcoholic controls P = 0.76, for alcoholic controls versus Wernicke’s encephalopathy P = 0.20, for alcoholic controls versus Korsakoff’s psychosis P = 0.0003, for Wernicke’s encephalopathy versus Korsakoff’s psychosis P = 0.008; Table 2 and Fig. 2B]. The degree of MD (r^2 = 0.55, P = 0.004) and AP (r^2 = 0.38, P = 0.03) atrophy correlated with the duration of Wernicke’s encephalopathy as did the combined atrophy of these midline nuclei (r^2 = 0.54, P = 0.004; Fig. 2D). Atrophy of the MD and AP correlated with the degree of thalamic atrophy (r^2 = 0.40, P = 0.0009) and

<table>
<thead>
<tr>
<th>Variable</th>
<th>Controls (n = 6)</th>
<th>Alcoholic controls (n = 5)</th>
<th>WE (n = 5)</th>
<th>WE and KP (n = 8)</th>
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<td>MM volume</td>
<td>90 ± 30</td>
<td>80 ± 20 (89%)</td>
<td>70 ± 60 (78%)</td>
<td>30 ± 15 (33%)*</td>
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<tr>
<td>MM number</td>
<td>53 ± 18</td>
<td>52 ± 11 (98%)</td>
<td>28 ± 19 (53%)*</td>
<td>17 ± 14 (32%)*</td>
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<td>AP volume</td>
<td>300 ± 50</td>
<td>280 ± 30 (93%)</td>
<td>260 ± 20 (87%)</td>
<td>190 ± 50 (63%)*</td>
</tr>
<tr>
<td>AP number</td>
<td>120 ± 22</td>
<td>121 ± 9 (100%)</td>
<td>103 ± 17 (86%)</td>
<td>56 ± 22 (47%)*</td>
</tr>
<tr>
<td>MD volume</td>
<td>1600 ± 200</td>
<td>1600 ± 150 (100%)</td>
<td>1400 ± 300 (88%)</td>
<td>900 ± 350 (56%)*</td>
</tr>
<tr>
<td>MD number</td>
<td>636 ± 44</td>
<td>643 ± 37 (100%)</td>
<td>330 ± 292 (52%)*</td>
<td>227 ± 157 (36%)*</td>
</tr>
</tbody>
</table>

Volumes are given in mm^3 and neuronal number is ×10 000; the percentage is that of the control mean. KP = Korsakoff’s psychosis; WE = Wernicke’s encephalopathy. *P < 0.05 from the non-highlighted diagnostic groups using ANOVA with Fisher’s protected t test.
The degree of neuronal loss in the diencephalic limbic relay nuclei

MM hypothalamic nucleus

The estimated number of neurons in the MM of all alcoholics with Wernicke’s encephalopathy was significantly less than that of alcoholic and non-alcoholic controls \[ F(3,20) = 8.3, P = 0.0009 \]; post hoc \( P \) values for controls versus Korsakoff’s psychosis \( P = 0.0004 \), for controls versus Wernicke’s encephalopathy alone \( P = 0.02 \), for non-alcoholic controls versus alcoholic controls \( P = 0.90 \), for alcoholic controls versus Wernicke’s encephalopathy \( P = 0.03 \), for alcoholic controls versus Korsakoff’s psychosis \( P = 0.0009 \), for Wernicke’s encephalopathy versus Korsakoff’s psychosis \( P = 0.22 \); Table 2) and positively correlated with MM volume \( r^2 = 0.36, P = 0.003 \) but not with the duration of Wernicke’s encephalopathy \( r^2 = 0.07, P = 0.4 \). There was no significant difference between the estimated number of MM neurons in alcoholics with Wernicke’s encephalopathy and those with additional Korsakoff’s psychosis (Table 2).
**MD thalamic nucleus**

There was a significant reduction in the estimates of total neuron number in the MD of all alcoholics with Wernicke’s encephalopathy compared with alcoholic and non-alcoholic controls \([F(3,20) = 12, P < 0.0001, post hoc P\) values for: controls versus Korsakoff’s psychosis \(P = 0.0001\), for controls versus Wernicke’s encephalopathy alone \(P = 0.01\), for non-alcoholic controls versus alcoholic controls \(P = 0.50\), for alcoholic controls versus Wernicke’s encephalopathy \(P = 0.003\), for alcoholic controls versus Korsakoff’s psychosis \(P < 0.0001\), for Wernicke’s encephalopathy versus Korsakoff’s psychosis \(P = 0.17; Table 2\), with the degree of atrophy correlating with neuronal loss \((r^2 = 0.61, P < 0.0001)\) but not with the duration of Wernicke’s encephalopathy \((r^2 = 0.14, P = 0.2)\). There was no significant difference between the estimated number of MD neurons in alcoholics with Wernicke’s encephalopathy and those with additional Korsakoff’s psychosis (Table 2).

**AP thalamic nucleus**

The estimated number of neurons in the AP of alcoholics with Korsakoff’s psychosis was significantly reduced \([F(3,20) = 15, P < 0.0001; post hoc P\) values for: controls versus Korsakoff’s psychosis \(P < 0.0001\), for controls versus Wernicke’s encephalopathy alone \(P = 0.21\), for non-alcoholic controls versus alcoholic controls \(P = 0.43\), for alcoholic controls versus Wernicke’s encephalopathy \(P = 0.06\), for alcoholic controls versus Korsakoff’s psychosis \(P < 0.0001\), for Wernicke’s encephalopathy versus Korsakoff’s psychosis \(P = 0.0015; Table 2\), with the neuronal loss correlating with the degree of atrophy \((r^2 = 0.65, P < 0.0001)\) but not with the duration of Wernicke’s encephalopathy \((r^2 = 0.09, P = 0.3)\). The unexpected and striking neuronal loss in the AP of alcoholics with Korsakoff’s psychosis was further analysed to see if single section sampling could differentiate alcoholics with Korsakoff’s psychosis. For each case the section with the greatest neuronal density was chosen for analysis. Using this single section sampling, there was no significant difference in the maximum AP neuronal density between any of the groups \(5000 \pm 1200\) neurons/mm\(^3\), alcoholic controls \(6400 \pm 1600\), Wernicke’s encephalopathy alone \(6000 \pm 1600\), Korsakoff’s psychosis \(5200 \pm 1700\); \(F(3,19) = 1.06, P > 0.05\). This suggests that in many cases substantial tissue atrophy is associated with this cell loss, with the sequelae that the cellular density remains relatively constant. Thus, neuronal density measures of the AP nucleus require regional volume correction to adequately evaluate neuronal loss in Korsakoff’s psychosis, with regional atrophy and substantial neuronal loss in the AP distinguishing alcoholics with Korsakoff’s psychosis from those with Wernicke’s encephalopathy (Table 2).

**Discussion**

There has been considerable debate on the differentiating lesions responsible for Korsakoff’s psychosis in chronic alcoholics. This question has been confounded by poor recognition of the clinical signs of Wernicke’s encephalopathy in the majority of chronic alcoholics and the neuropathological similarity between alcoholics with Wernicke’s encephalopathy alone or in association with Korsakoff’s psychosis (Harper et al., 1986; Naidoo et al., 1991; Caine et al., 1997). Thirty to eighty per cent of chronic alcoholics have clinical or biochemical signs of thiamine deficiency (Darnton-Hill and Truswell, 1990) and all alcoholics with Wernicke’s encephalopathy caused by thiamine deficiency have significant periventricular neuropathology (Torvik, 1987; Victor et al., 1989). Therefore, one of the first difficulties in identifying differentiating brain lesions for alcoholic Korsakoff’s psychosis is selection of adequate cases for comparison, namely those with Wernicke’s encephalopathy but without amnesia. We used recently validated, highly sensitive and specific criteria (Caine et al., 1997) to identify our sample of patients and therefore are confident in their clinicopathological classification. Furthermore, we have recently reported the degree of neurodegeneration in many other brain regions for the majority of these cases. This includes regional tissue volume analysis of the entire brain (Kril et al., 1997) and quantification of the degree of neurodegeneration in the hippocampus (Harding et al., 1997) and dorsolateral prefrontal cortex (Kril et al., 1997), in addition to the cholinergic basal forebrain (Cullen et al., 1997), noradrenergic locus coeruleus (Halliday et al., 1992), serotoninergic raphe (Halliday et al., 1993) and the vasopressin-containing neurons of the hypothalamus (Harding et al., 1996). No difference was found between amnesic and non-amnesic alcoholics with Wernicke’s encephalopathy in any of these regions.

A recent neuroimaging study of reformed alcoholics with Korsakoff’s psychosis showed widespread decline in glucose metabolism (Paller et al., 1997) suggesting that cortical dysfunction contributes to the amnesia of Korsakoff’s psychosis. However, non-amnesic alcoholics with Wernicke’s encephalopathy were not analysed and tissue volume changes not accounted for. We have identified previously significant atrophy of the white matter, amygdala, hippocampus and thalamus with sparing of all other brain regions in alcoholics with Wernicke’s encephalopathy (Harding et al., 1997; Kril et al., 1997). These widespread volume changes were not exclusive to alcoholics with Korsakoff’s psychosis suggesting slowing of information processing in all alcoholics with Wernicke’s encephalopathy and providing little evidence for a primarily cortical substrate for any additional amnesia.

The severe amnesia of alcoholic Korsakoff’s psychosis has most frequently been ascribed to mamillary body damage. This region is where the pathology of Wernicke’s encephalopathy is most consistently found (Torvik, 1987; Kril, 1996). However, mamillary body atrophy also occurs in alcoholics without Korsakoff’s psychosis and there is no correlation with the degree of memory impairment (Charness and DeLaPaz, 1987; Davila et al., 1994; Shear et al., 1996; Estruch et al., 1998). Of the thalamic regions, the MD is
The diencephalon in alcoholic amnesia

Fig 3 Photomicrographs of the right MD. A–C were all stained with haematoxylin and eosin, and photographed at the same magnification at similar levels. D–F are higher magnifications of the sections adjacent to A–C, stained with cresyl violet and photographed at the same magnification. (A) The normal architecture of the MD and surrounding structures in a control, for comparison with B and C. (B) A representative slide from an alcoholic with Wernicke’s encephalopathy. Most of these alcoholics had significant vascular and parenchymal neuropathology, including petechial haemorrhages (arrows), blood vessel abnormalities and myelin changes. These changes were confined to the MD with the surrounding intralaminar nuclei well preserved. (C) A representative slide from an alcoholic with Korsakoff’s psychosis. Similar vascular changes were observed in the MD of these alcoholics, including blood vessel abnormalities, myelin changes, parenchymal changes and obvious tissue shrinkage. Again these changes appeared confined to the MD. (D) Normal neuronal and glial configuration for comparison with E and F. (E) An alcoholic with Wernicke’s encephalopathy. In general there was a significant reduction in the number of visible neurons with an associated gliosis. (F) This slide illustrates similar neuronal changes that were observed in alcoholics with Korsakoff’s psychosis with a reduction in the number of visible neurons and an increase in glial density.
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Fig 4 Photomicrographs of the right AP. A–C were all stained with haematoxylin and eosin, and photographed at the same magnification at similar levels. D–F are higher magnifications of the sections adjacent to A–C, stained with cresyl violet and photographed at the same magnification. (A) The normal architecture of the AP and surrounding structures in a control for comparison with B and C. (B) An alcoholic with Wernicke’s encephalopathy. In some alcoholics there were limited changes in the AP. (C) An alcoholic with Korsakoff’s psychosis. There were often blood vessel abnormalities, myelin and parenchymal changes, and shrinkage of the AP. (D) Normal neuronal and glial configuration for comparison with E and F. (E) The slide shows changes observed in an alcoholic with Wernicke’s encephalopathy, including gliosis without apparent neuronal loss. The scale bar = 100 µm. (F) An alcoholic with Korsakoff’s psychosis. In most of these alcoholics there was a visible reduction in the number of neurons and a further increase in the density of glia.

commonly identified radiologically (McDowell and LeBlanc, 1984; Roche et al., 1988; Shimamura et al., 1988; Charness, 1993) and neuropathologically (Mair et al., 1979; Torvik, 1985; Mayes et al., 1988; Victor et al., 1989) as damaged in alcoholics with Wernicke’s encephalopathy. Our results indicate that neuronal loss is most severe in the MM with cell loss in both nuclei occurring prior to tissue atrophy. This suggests a primary insult to MM and MD neurons rather than atrophy due to deafferentation. As alcoholics with similar durations of Wernicke’s encephalopathy, regardless
of the presence of amnesia, had the same degree of MM and MD damage, we suggest that the loss of these diencephalic regions is not sufficient to produce the permanent amnesia found in alcoholic Korsakoff’s psychosis. Instead we propose that additional neuronal loss in the AP is required for amnesia to develop in these alcoholic.

In humans, the MM comprises most of the mamillary body, receiving hippocampal afferents via the fornix and projecting to the anterior thalamic nuclei (Sziklas and Petrides, 1998). Apart from MM afferents, the AP also receives direct hippocampal input via the fornix (Aggleton et al., 1986) and has reciprocal connections with cingulate cortices and the rostral portion of the thalamic reticular nucleus (Velasco et al., 1989; Velayos et al., 1989; Gonzalo-Ruiz et al., 1997). The MD receives more widespread afferents from the amygdala, basolateral amygdala, hippocampus, substantia nigra pars reticulata and dorsolateral tegmental area, and has reciprocal projections with the prefrontal and piriform cortices and thalamic reticular nucleus (Aggleton and Mishkin, 1984; Ilnsky et al., 1985; Russchen et al., 1987; Churchill et al., 1996; Gritti et al., 1998; Kuroda et al., 1998). Therefore, while the AP is considered an integral part of the ‘extended hippocampal system’ (Aggleton and Saunders, 1997), the MD is thought to modulate frontal activation, attention and sleep-wakefulness (Gritti et al., 1998; Lugaresi et al., 1998; Velayos et al., 1998).

All alcoholics with acute Wernicke’s encephalopathy and MM pathology have a characteristic confusion (disorientation in time and place), inattentiveness and lethargy. In our cases the lethargy improved quickly following thiamine administration, while the disorientation and inattentiveness took up to 3–4 weeks to resolve. This occurred even in alcoholics with repeated episodes of clinical Wernicke’s encephalopathy and substantial mamillary body damage but without persistent amnesia. Lesioning of the mamillary bodies in laboratory animals suggests this region contributes to spatial learning and memory, depending on the level of task difficulty and the degree of damage, but does not affect visual discrimination and recognition memory (Aggleton et al., 1995; Béragochéa and Jaffard, 1995; Neave et al., 1997; Parker and Gaffan, 1997b; Sziklas and Petrides, 1998). Long-term follow-up in non-human (Zola-Morgan et al., 1989) and human (Victor et al., 1989; Dusoir et al., 1990) primates suggests that many of the deficits caused by mamillary body lesioning are transient rather than permanent.

In mice, similar behavioural and neuroanatomical deficits are elicited by chronic ethanol consumption (Béragochéa et al., 1987, 1995) and can be reversed by methyl β-carboline-3-carboxylate, a benzodiazepine antagonist (Béragochéa et al., 1995), supporting the concept of functional recovery. Such functional recovery may depend on modulation of non-damaged AP neurons via direct hippocampal and cingulate pathways or possibly via reticular thalamic feedback.

Infarction of the MD is characterized by confusion and inattentiveness, with lethargy in severe cases (Cole et al., 1992). Patients often have more permanent behavioural changes becoming apathetic and introverted with an amnestic syndrome that can either persist or resolve depending on the extent of pathology (Kritchnevsky et al., 1987; Cole et al., 1992; Peru and Fabbro, 1997; Shuren et al., 1997). Lethargy, inattentiveness and an inability to generate EEG sleep patterns are associated with severe MD degeneration (often >90% neuronal loss) in fatal familial insomnia (Lugaresi et al., 1998). This is consistent with the changes in EEG activity following experimental MD lesioning in cats (Marini et al., 1989) and recent studies of attention and arousal in humans (Portas et al., 1998), and is further supported by a case report of an alcoholic with resolving MD lesions associated with improvement in attention and arousal (Donnal et al., 1990). Thus, MD lesions have been associated with the mental state changes that are characteristic of clinical Wernicke’s encephalopathy.

There have been only a few cases described with damage to the anterior thalamus sparing the MD (Daum and Ackermann, 1994; Schnider et al., 1996). These patients have unilateral right-sided lesions that cause an amnesia similar to that seen in alcoholic Korsakoff’s psychosis. Thus, damage to the anterior thalamus is sufficient to produce permanent deficits in episodic memory, supporting previous studies emphasizing the importance of this region for the memory deficit in alcoholic Korsakoff’s psychosis (Mair et al., 1979; Mayes et al., 1988; Kopelman, 1995). These findings are also consistent with the severe memory deficits caused by lesions of the anterior thalamic nuclei in laboratory animals (Aggleton and Sahgal, 1993; Aggleton et al., 1995; Aggleton and Saunders, 1997; Parker and Gaffan, 1997a), with the degree of memory dysfunction related to the extent of the lesion (Aggleton et al., 1996). In our cases, the AP was not affected in isolation in the alcoholics with Korsakoff’s psychosis, but was always damaged along with the MM and MD.

These findings are consistent with those of Victor and colleagues who highlighted the correlation between MD neuropathology and Korsakoff’s psychosis (Victor et al., 1989). However, we provide further quantitative evidence of the importance of additional AP degeneration in cases in which we have discounted other neuronal deficits. All previous studies concentrating on the tissue pathology underlying alcoholic Korsakoff’s psychosis have identified the anteromedial thalamic region as the site of differential pathology (Mair et al., 1979; Mayes et al., 1988; Victor et al., 1989). These studies describe the visual changes in cellular densities as being the most striking pathology observed in amnesic alcoholics, with two of these studies concentrating on the increased periventricular gliosis in the region of the paraaenial thalamic nucleus (Mair et al., 1979; Mayes et al., 1988). There is considerable species differences in thalamic architecture, with the paraaenial nucleus in humans only a remnant of the cellular structure found in rodent species (Bentivoglio et al., 1993). In rodents the nucleus receives considerable innervation from the hypothalamus and lateral septum, but in humans it is
represented by only a thin band of cells on the anterior and dorsal aspect of the MD and intralaminar thalamic nuclei (Bentivoglio et al., 1993). Cytoarchitecturally the neurons of the parataenial nucleus are indistinguishable from those of the MD and these regions fuse posteriorly (Bentivoglio et al., 1993). The increased gliosis observed in this region may relate to its substantial hypothalamic innervation and the hypothalamic degeneration sustained in all alcoholics with Wernicke’s encephalopathy, especially as neuronal loss was not a feature of the amnesic cases in these previous studies (Mair et al., 1979; Mayes et al., 1988). It is of note that Victor and colleagues (Victor et al., 1989) identified similar periventricular gliosis in all their alcoholics with Wernicke’s encephalopathy and determined that neuronal loss in the MD was a more important factor influencing clinical amnesia. Our results are in agreement with those of Victor and colleagues (Victor et al., 1989), although these neurons would have been included in our measures of MD neuronal loss because of their position and cytoarchitectural similarity in humans.

To accurately identify the degree of neuronal degeneration, volumetric as well as density measures are required. We have performed such measurements for the first time in alcoholics in regions considered important for adequate memory function. All previous studies have analysed changes in neuronal densities either visually or by quantification (Mair et al., 1979; Mayes et al., 1988; Victor et al., 1989; Belzunegui et al., 1995). However, considerable atrophy of brain tissue is a well-documented sequela of chronic alcohol abuse, even in the absence of Wernicke’s encephalopathy (Kril and Halliday, 1999), and therefore changes in cellular density should be interpreted with some caution. It is of interest that the neuronal loss in the MM and MD appears somewhat different to that observed in the AP, a result also highlighted by Victor and colleagues (Victor et al., 1989). Neuronal loss occurs prior to regional atrophy in the MM and MD while regional atrophy occurs in concert with the neuronal degeneration in the AP. Thus, changes in neuronal density are less obvious in the AP in many cases. This was substantiated by our evaluation of single section density changes in the AP which showed that neurodegeneration could not be accurately determined using density measures, and the use of volumetric correction of neuronal densities was therefore an essential tool in determining the degree of neuronal loss, particularly in the AP.

The data from the present study provide the following picture. Following thiamine deficiency, neuronal damage appears prior to significant volume reduction in the diencephalic limbic relay nuclei, and further loss of afferent innervation over time contributes to the relationship observed between tissue volume and duration of Wernicke’s encephalopathy. However, even though the degree of atrophy in these diencephalic regions relates to the duration of Wernicke’s encephalopathy, Korsakoff’s psychosis was not the inevitable outcome in these alcoholics with Wernicke’s encephalopathy (Ryan and Butters, 1980). Neurodegeneration of the AP was the only consistent lesion found in alcoholics with Korsakoff’s psychosis which differentiated them from other alcoholics with Wernicke’s encephalopathy. In fact, AP degeneration is the only differentiating lesion we have so far observed in these alcoholic cases with Korsakoff’s psychosis (Halliday et al., 1992, 1993; Harding et al., 1996, 1997; Cullen et al., 1997; Kril et al., 1997). We therefore suggest that neurodegeneration of the AP is critical for their amnesia.

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References


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