

The occurrence of depressive symptoms in the preclinical phase of AD

A population-based study

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Article abstract—*Objective:* To examine preclinical depressive symptoms 3 years before the diagnosis of AD. *Methods:* The authors compared incident AD patients and nondemented individuals in terms of baseline mood- and motivation-related symptoms of depression, and assessed whether depressive symptoms in preclinical AD are related to self-perceived memory problems. Participants came from a population-based longitudinal study on aging and dementia in Stockholm, Sweden. The sample consisted of 222 persons older than 74 years who were followed for a 3-year interval. Thirty-four individuals had developed AD at follow-up, whereas 188 remained nondemented. Dementia diagnosis was made according to the criteria of the Diagnostic and Statistical Manual of Mental Disorders, 3rd edition, revised. Depressive symptoms were assessed by the Comprehensive Psychopathological Rating Scale. *Results:* The incident AD patients had more depressive symptoms than the nondemented persons at baseline. There was a dominance of motivation-related symptoms of depression (e.g., lack of interest, loss of energy, concentration difficulties) in preclinical AD. This association remained when adjusting for subjective memory complaints. *Conclusions:* Depressive symptoms are elevated preclinically in AD, and this elevation is not merely a by-product of self-perceived cognitive difficulties. Thus, depressive symptoms may be part of the preclinical phase in AD. **Key words:** Preclinical AD—Depressive symptoms—Subjective memory complaints.

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Depressive symptoms occur more frequently in demented compared with nondemented older adults.^{1,2} The prevalence of depressive disorders in patients with AD varies between 15 and 50%,^{2,4} and in nondemented elderly people it ranges between 1 and 10%.^{5–7} The relationship between depressive disorders and dementia is not fully understood. Some researchers have argued that depression is a risk factor for AD.^{8,9} A substantial proportion of those nondemented elderly individuals who experience a first episode of depression in late life will develop a dementia syndrome within 3 to 8 years after the onset of depression.^{9,10} Other investigators have suggested that depressive reactions may be an early sign of an impending dementia disease rather than a risk factor for AD.^{11,12} Indeed, given that AD has a rather long preclinical period, the observation that depressive episodes several years before the clinical onset of dementia is associated with AD may be interpreted as an early sign rather than as a risk factor.¹³ However, some researchers have observed a relationship between depression and dementia only for late-onset cases.^{14,15}

Many important issues are still unresolved concerning the relationship between depression and dementia. For example, little is known about depressive symptomatology in persons who will develop AD because

research has focused on symptom expression in clinical samples using retrospective data^{16,17} or has relied on information from caregivers and other secondary informants.^{18,19} The current study provides information about depressive symptoms in the preclinical phase of AD using prospective data.

In addition to examining the specific symptoms for major depression cited in the Diagnostic and Statistical Manual of Mental Disorders, 3rd edition, revised (DSM-III-R),²⁰ we explored two symptom constellations that have been termed mood-related (i.e., dysphoria, appetite disturbance, feelings of guilt, and thoughts of death/suicidal ideation) and motivation-related (i.e., lack of interest, psychomotor change, loss of energy, concentration difficulties) symptoms.²¹ Knowledge pertaining to whether there is a predominance of either mood- or motivation-related symptoms of depression in preclinical AD may shed light on the nature of the depressive signs. Specifically, if mood-related symptoms dominate in preclinical AD, this may reflect in part emotional reactions to self-perceived cognitive difficulties and, possibly, insight into an emerging disease process. By contrast, the existence of motivation-related symptoms may be independent of self-perceived cognitive decline and may reflect disease-related changes in brain regions critical to the allocation of

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attentional energy. To address this issue, we also determined the relationship between self-perceived memory problems and depressive symptoms.

Thus, three specific research questions were asked: 1) Do individuals who will develop AD present with an elevation in depressive signs and symptoms compared with control subjects 3 years before diagnosis? 2) If elevated depressive symptoms could be demonstrated, is there a predominance of either mood- or motivation-related symptoms? 3) Are these symptoms related to perceived decline in cognitive functioning or are they independent of subjectively experienced cognitive problems?

Methods. *Participants.* The original sample was taken from all the inhabitants age 75 years and older in the Kungsholmen parish of Stockholm, Sweden (2,368 individuals), who were included in a population survey on aging and dementia. A more detailed description of the population and methods used has been reported elsewhere.^{22,23} At baseline, 1,810 individuals from the whole study population were administered a questionnaire, which included the Mini-Mental State Examination (MMSE),²⁴ to detect suspected dementia patients. All those with an MMSE score less than 24 (n = 314) and a random sample, stratified by age and gender, of those with an MMSE score more than 23 (n = 354) were assessed with extensive medical, neurologic, and psychiatric examinations; social and family interviews; laboratory blood analyses; and a comprehensive cognitive test battery. At follow-up, all participants were invited back to be examined approximately 3 years later. The same protocol was administered at this time. The Kungsholmen Project has been approved by the ethics committee of the Karolinska Institute, Sweden, and informed consent was obtained from all participants after details of the procedure were fully explained.

The diagnostic criteria and procedure used to reach the clinical diagnosis of dementia, as well as type of dementia, at baseline and follow-up is described in detail by Fratiglioni et al.,²³ and involved multiple steps: In the first step, a preliminary diagnosis was made after a common discussion among geriatricians who had examined the participants and reviewed their social and family history. Step 2 involved a second preliminary diagnosis of all participants by a physician expert in dementia. In step 3, the two preliminary diagnoses were compared, and patients with discordant diagnoses were reviewed again by the physicians to ascertain causes of agreement and disagreement. This eliminated most of the discordant diagnoses. In those patients in whom disagreement persisted, the final diagnosis was made by a supervising physician. This process yielded a diagnosis of dementia and dementia type according to the DSM-III-R.²⁰ At baseline, there were 225 persons who received a dementia diagnosis, whereas 443 were found to be nondemented. The current study focuses on those who were nondemented at the baseline assessment.

Of the 443 persons in the nondemented group, 45 were excluded from the current study because of incomplete psychiatric or cognitive data. Using data from the baseline assessment, we also eliminated 46 persons with MMSE scores less than 24,²⁵ 20 persons with psychiatric disease (e.g., depressive disorders, psychosis), 17 persons with a history of stroke, and three persons with PD. Of the re-

maining 312 participants, 34 were diagnosed with probable or possible AD after approximately 3 years of follow-up (mean, 3.08 years; SD, 0.58). In addition, nine individuals were diagnosed with dementia other than AD (e.g., vascular dementia, mixed vascular, unspecified), 52 had died, and 29 dropped out (e.g., refused, moved, could not be located). Thus, the final sample consisted of 34 incident AD and 188 nondemented persons.

Measures. *Depressive signs and symptoms.* A psychiatric examination was administered by physicians using the Comprehensive Psychopathological Rating Scale (CPRS).²⁶ The CPRS is a structured psychiatric instrument with both directed questions and observations related to a variety of conditions, including depressive, anxiety, and sleep disorders, as well as psychotic symptoms. Symptoms and signs were rated on a 6-point scale, with definitions at every second step.²⁶ Specific items in the CPRS match the symptoms for major depression according to the DSM-III-R.²⁰ Thus, the relevant CPRS items were reviewed to examine whether a particular DSM-III-R symptom of depression was present. The depressive symptoms were separated into two categories, reflecting either mood- or motivation-related disturbance.²¹ The mood symptoms included dysphoria, appetite disturbance, feelings of guilt, and thoughts of death/suicidal ideation. The motivational symptoms included lack of interest, psychomotor change, loss of energy, and concentration difficulties. Although sleep disturbance is not part of either symptom cluster, it was included in the current analyses because it constitutes a symptom of major depression according to the DSM-III-R. In addition, the item assessing subjective memory complaints in the CPRS (range, 0 to 6 points) was included in the analyses.

Information was gathered at follow-up concerning prior psychiatric disorders, contact with health care professionals for psychiatric symptoms, and antidepressant drug treatment. None of the incident AD patients had a history of depression or other psychiatric disorder.

Background variables. In addition to age, gender, and years of education, information regarding the participants' level of global cognitive functioning and functional ability was gathered. Global cognitive functioning was indexed by the MMSE.²⁴ A Swedish version of the test was administered according to standardized procedures, and the total score was a maximum of 30 points. Functional ability was assessed using the Katz activities of daily living index.²⁷ This measure inquires about the person's ability to perform tasks such as bathing, dressing, going to the toilet, transferring, continence, and feeding. The scale ranges from 1 (independent) to 6 (dependent).

Results. Background characteristics of the incident AD and nondemented participants at baseline are presented in table 1. A multivariate analysis of variance (MANOVA) revealed an overall effect of group status (Wilks' $\lambda = 0.75$, $F[1,220] = 18.38$, $p < 0.001$). Univariate analyses showed that the incident AD patients were older ($F[1,220] = 6.91$, $p < 0.01$), had lower MMSE scores ($F[1,220] = 59.81$, $p < 0.001$), and were more functionally impaired ($F[1,219] = 4.72$, $p < 0.05$) compared with the nondemented persons at baseline.

Baseline differences in depressive symptoms. *Mean-level analyses.* Table 2 displays the means and SDs for the depressive symptoms at baseline in the two groups.

Table 1 Baseline background characteristics of incident AD and nondemented persons

Variable	Diagnosis of AD 3 years later	
	Incident AD (n = 34), mean \pm SD	Nondemented (n = 188), mean \pm SD
Age, y	85.53 \pm 4.95	83.18 \dagger \pm 4.77
Gender, % female	91	80
Education, y	8.56 \pm 2.56	9.04 \pm 3.05
MMSE score	25.65 \pm 1.48	27.94 \ddagger \pm 1.61
ADL \S	1.46 \pm 0.94	1.21* \pm 0.51

* $p < 0.05$.

\dagger $p < 0.01$.

\ddagger $p < 0.001$.

\S Katz ADL index.²⁷ Due to missing data, n = 221 (34 incident AD patients and 187 nondemented persons).

MMSE = Mini-Mental State Examination²⁴; ADL = activities of daily living.

MANOVA indicated that the incident AD patients had more depressive symptoms at baseline than their nondemented counterparts (Wilks' $\lambda = 0.935$, $F[1,220] = 5.06$, $p < 0.01$). Interestingly, at the level of the symptom clusters, the incident AD patients had more motivation-related symptoms than the nondemented persons ($F[1,220] = 15.00$, $p < 0.001$), although the two groups did not differ reliably regarding mood-related symptoms ($F[1,220] = 2.90$, $p > 0.05$).

With respect to the individual motivation-related symptoms, the incident AD patients reported more lack of interest ($F[1,220] = 16.08$, $p < 0.001$), loss of energy ($F[1,220] = 4.34$, $p < 0.05$), and concentration difficulties ($F[1,220] = 4.26$, $p < 0.05$) compared with nondemented

persons. Although there was no overall group difference in mood-related symptoms, the incident AD group had more thoughts of death than the control subjects ($F[1,220] = 11.12$, $p < 0.001$). The groups did not differ in the additional depressive symptom of sleep disturbance ($p > 0.10$). Lastly, the incident AD patients complained more about memory problems than the nondemented persons ($F[1,220] = 10.42$, $p < 0.001$; see table 2).

Logistic regression analyses. To determine whether the higher frequency of depressive symptoms in preclinical cases of AD was independent of age, gender, and education, a series of logistic regression analyses was carried out. Two types of regressions were conducted. In the first model, age, gender, and education were entered as covariates, and the two clusters of depressive symptoms were then entered in stepwise fashion. Table 3 shows that individuals who were going to develop AD had more motivation-related symptoms than those who remained nondemented, although there was no significant group difference for mood-related symptoms. In model 2, those individual symptoms of depression for which there were significant group differences were entered as predictors, using the same covariates. As shown in table 3, this analysis revealed that two symptoms—lack of interest and thoughts of death—were reliable predictors of dementia status at follow-up.

Given that 1) the two groups differed in subjective memory complaints and 2) self-perceived cognitive problems may be related to depressive symptoms in preclinical AD, we repeated the two models, entering subjective memory complaints between the covariates and the depressive symptoms in the regressions. These analyses showed that, although subjective memory complaints at baseline predicted dementia status at follow-up (odds ratio, 1.46; 95% CI, 1.08 to 1.97; $p < 0.05$), the results concerning the predictive power of the depressive symptoms remained unchanged (see table 3).

Discussion. We found that persons who would develop AD during a 3-year interval had more depressive symptoms at baseline assessment than persons who remained nondemented at follow-up. Although elevated depressive symptoms have been demonstrated in clinically verified AD patients,^{1,3,4} and although depression has emerged as a risk factor for AD in studies using retrospective data,^{8,17} the result of an increase in depressive symptoms demonstrated prospectively in preclinical AD patients from the population at large constitutes a novel finding. It is important to note, however, that the increase of depressive symptoms in the incident AD patients was relatively small, and that none of the participants received a diagnosis of clinical depression at baseline.

There was converging evidence for a dominance of motivation-related over mood-related symptoms of depression in the preclinical phase of AD. First, the mean-level analysis revealed an overall difference in motivational symptoms between the incident AD patients and the control group. Reliable group differences were seen for lack of interest, loss of energy, and concentration difficulties. By contrast, for the mood symptoms the overall group difference did not

Table 2 Means and SDs in baseline depressive symptoms and subjective memory complaints of incident AD patients and nondemented persons

Variable	Diagnosis of AD 3 years later	
	Incident AD (n = 34), mean \pm SD	Nondemented (n = 188), mean \pm SD
Mood factor	1.18 \pm 2.09	0.69 \pm 1.42
Dysphoria	0.35 \pm 0.66	0.21 \pm 0.46
Appetite disturbance	0.24 \pm 0.61	0.26 \pm 0.72
Feelings of guilt	0.24 \pm 0.50	0.15 \pm 0.45
Thoughts of death	0.35 \pm 0.88	0.07 \ddagger \pm 0.33
Motivation factor	1.38 \pm 1.95	0.55 \ddagger \pm 0.95
Lack of interest	0.38 \pm 0.74	0.08 \ddagger \pm 0.31
Psychomotor change	0.06 \pm 0.34	0.02 \pm 0.22
Loss of energy	0.53 \pm 1.02	0.26* \pm 0.63
Concentration difficulties	0.41 \pm 0.78	0.19* \pm 0.52
Sleep disturbance	1.38 \pm 1.67	1.17 \pm 1.46
Memory complaints	1.47 \pm 1.29	0.79 \ddagger \pm 1.10

* $p < 0.05$.

\ddagger $p < 0.001$.

Table 3 Logistic regression analyses for predicting group status at follow-up

Variable	Odds ratio adjusted for age, gender, and education	95% CI	p Value	Odds ratio adjusted for age, gender, education, and memory complaints	95% CI	p Value
Model 1						
Mood factor	1.20	0.97–1.48	0.087	1.15	0.92–1.44	0.209
Motivation factor	1.53	1.17–2.00	0.002	1.40	1.04–1.89	0.025
Model 2						
Lack of interest	3.03	1.47–6.24	0.003	2.72	1.31–5.67	0.007
Thoughts of death	2.30	1.13–4.68	0.021	2.14	1.01–4.54	0.046

approach significance, and the incident AD group showed a reliable increase in only one symptom—thoughts of death. Second, controlling for demographic variables in a logistic regression analysis, the cluster of motivational symptoms predicted diagnostic status at follow-up, whereas the mood cluster did not. Lastly, although both lack of interest and thoughts of death emerged as significant predictors of group membership in the logistic regression analysis focusing on individual symptoms, the predictive power of lack of interest exceeded that of thoughts of death. An important point to note is that, although the incident AD patients complained more about memory problems than the control subjects, the relationship between depressive symptoms and diagnostic status remained unchanged regardless of whether subjective memory complaints were controlled statistically.

Thus, the current pattern of findings suggests that depressive symptoms in the preclinical phase of AD may be related mostly to motivational factors rather than to factors typically associated with depression (e.g., dysphoria). To the extent that symptoms such as lack of interest, loss of energy, and concentration difficulties are common in the normal older population,^{5,28,29} these symptoms may be overlooked easily as early markers of an emerging dementing disease.

The motivational symptoms of depression are primarily cognitively loaded, and have been linked to the individual's basic processing resources, such as the ability to focus attention on the task at hand, while closing out irrelevant information.^{30,31} For example, it has been demonstrated that motivation-related, but not mood-related, symptoms of depression predict cognitive performance in clinically nondepressed and nondemented older adults.³⁰ It is well established that deficits in memory and other cognitive functions are common during the preclinical phase of AD.^{32,33} The current finding that the incident AD patients had lower MMSE scores at baseline than the control subjects confirms that cognitive impairment occurs before the time at which a clinical diagnosis of AD is rendered. Cognitive deficits in preclinical AD have been linked to early changes in both limbic and neocortical brain structures.^{34,35} Following this line of reasoning, the observation that the incident AD patients showed elevated motivational symptoms of depression 3 years

before diagnosis may reflect early changes in brain regions critical to the ability to allocate and to sustain attentional energy.

At the level of the individual symptoms, lack of interest and thoughts of death predicted group status at follow-up. The finding that thoughts of death at baseline was related to incidence of AD is particularly intriguing because this symptom belongs to the mood cluster that, overall, was unrelated to incidence of AD. One possibility is that the elevation of this symptom reflects weariness of life rather than concrete death wishes among the incident AD patients. Note also that a major reason for the group difference observed for thoughts of death was the low frequency of this symptom in the nondemented persons. Future research is required to assess the replicability of this finding.

As noted, the increase of depressive symptoms in preclinical AD was unrelated to subjective memory complaints. This result suggests that the elevation of symptoms was not a mere reaction to self-perceived cognitive difficulties. The fact that the effects were not mediated through subjective memory problems is interesting in light of the observed dominance of motivation-related symptoms. Specifically, had there been an association between memory complaints and depressive symptoms, this association may have reflected insight into an emerging dementia disease and resulted in mood changes. In this way, the lack of relationship between memory complaints and depressive symptoms observed in this study is consistent with the view that mood-related symptoms are relatively uncommon in preclinical AD.

An interesting question concerns whether the balance between mood- and motivation-related symptoms of depression changes during the pathogenesis of AD. In clinical AD, there is evidence that mood-related symptoms dominate in mild to moderate stages, and that motivation-related symptoms dominate in severe stages of the disease.²¹ Comparing these findings with those of the current study, an interesting picture emerges: Although mood-related symptoms of depression may dominate in early clinical AD, there is a dominance of motivation-related symptoms both preclinically and in severe stages of the disease. This intriguing pattern appears to reflect the facts that the preclinical cognitive deficits in AD are not strong enough to trigger marked mood-

related reactions, but also that a certain level of cognitive ability is required to feel or to express mood-related symptoms of depression.

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Clinicopathologic correlates in temporal cortex in dementia with Lewy bodies

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Article abstract—*Objective:* To address the relationship between dementia and neuropathologic findings in dementia with Lewy bodies (DLB) in comparison with AD. *Methods:* We evaluated the clinical presentation of autopsy-confirmed DLB in comparison with AD according to new Consortium on DLB criteria and compared the two conditions using quantitative neuropathologic techniques. This clinicopathologic series included 81 individuals with AD, 20 with DLB (7 “pure” DLB and 13 “DLB/AD”), and 33 controls. We counted number of LB, neurons, senile plaques (SP), and neurofibrillary tangles (NFT) in a high order association cortex, the superior temporal sulcus (STS), using stereologic counting techniques. *Results:* The sensitivity and specificity of Consortium on DLB clinical criteria in this series for dementia, hallucinations, and parkinsonism are 53% and 83%, respectively, at the patient’s initial visit and 90% and 68%, respectively, if data from all clinic visits are considered. In pathologically confirmed DLB brains, LB formation in an association cortical area does not significantly correlate with duration of illness, neuronal loss, or concomitant AD-type pathology. Unlike AD, there is no significant neuronal loss in the STS of DLB brains unless there is concomitant AD pathology (neuritic SP and NFT). *Conclusions:* The evaluation of new Consortium on DLB criteria in this series highlights their utility and applicability in clinicopathologic studies but suggests that sensitivity and specificity, especially at the time of the first clinical evaluation, are modest. The lack of a relationship of LB formation to the amount of Alzheimer-type changes in this series suggests that DLB is a distinct pathology rather than a variant of AD. **Key words:** Superior temporal sulcus—Stereology—Lewy bodies—Neuronal loss—Neurofibrillary tangles—Amyloid deposition—Dementia with Lewy bodies.

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Recent data suggest that dementia with Lewy bodies (DLB) represents the second most frequent cause of degenerative dementia in the elderly after AD.^{1–6} However, the relationship between DLB and AD continues to be confusing. The pathologic hallmarks of these conditions—senile plaques (SP) and neurofibrillary tangles (NFT) in AD, and LB in DLB—often coexist in the brain.^{3,7,8} Furthermore, differing diagnostic criteria variably segregate dementia brains into AD plus DLB or pure DLB categories.⁹ Many series thus include a mixture of patients with different degrees and overlap of both types of pathologic changes, complicating the task of comparing observations reported by different groups.

The underlying cause of dementia in DLB, which resembles the dementia of AD in many respects, remains unknown. Neurochemical assessments have found a profound deficiency of cortical acetylcholine activity in DLB even exceeding that seen in AD brains, suggesting a common pathologic involvement of the basal forebrain in these two conditions.^{10–12} The significance of abnormal intraneuronal inclusions in DLB brains, the so-called LB, remains uncertain. Furthermore, the relative contribution of LB and of concomitant AD changes in many of these brains to

dementia is poorly understood. Despite the frequent coexistence of LB and AD-related changes, the topographic distribution of LB in DLB does not match the distribution of Alzheimer-type pathologic changes. Cortical LB are preferentially present in the deeper layers of the frontal, cingulate, insular, and temporal cortices, whereas NFT develop most frequently in layers II, III, and V of high order association cortices^{13–15} as well as the entorhinal cortex, hippocampal formation, and amygdala. Whether these differences reflect unique and recognizable patterns of neuronal vulnerability in DLB remains unresolved. Furthermore, although it is well established that loss of neurons and synapses closely correlates with the progression of cognitive deficits in AD brains,¹⁶ less is known about the clinicopathologic correlations of these changes in DLB brains.^{17–19}

Overall, increasing evidence suggests that DLB and AD may be successfully discriminated from both clinical and pathologic perspectives. Recently the Consortium on DLB International Workshop proposed consensus guidelines to help clinical and neuropathologic efforts to further understand this type of dementia.²⁰ The unifying term “dementia with Lewy bodies” has been suggested.²⁰ Recurrent visual

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Table 1 Demographics of individuals studied

Characteristic	AD (n = 81)	DLB/AD (n = 13)	DLB (n = 7)	Control (n = 33)
Sex/no.	M/35 F/46	M/8 F/5	M/6 F/1	M/13 F/20
Age at death, y	78.5 ± 9.3	78.2 ± 9.2	77.9 ± 6.9	74.5 ± 16.61
Age at onset, y	69.2 ± 10	69.5 ± 10.1	68.7 ± 8.2	
Duration, y	9.3 ± 4.3	8.3 ± 3.9	9 ± 6.1	

Values are mean ± SD.

DLB = dementia with Lewy bodies.

hallucinations, spontaneous motor features of parkinsonism, and fluctuating cognition have been proposed as cardinal clinical features that predict DLB with high likelihood.²⁰ The Consortium on DLB recommended describing and staging the presence of LB to establish the pathologic diagnosis based on a brain sampling scheme and a semiquantitative scale consistent with the Consortium to Establish a Registry for AD (CERAD) and noting any coexisting pathologic lesions such as AD-related pathology. These consensus criteria now need to be tested with further clinicopathologic studies.

The present study aimed 1) to evaluate the new Consortium on DLB criteria in a clinicopathologic series, 2) to address whether or not LB accumulate with time in association cortices in DLB, and 3) to assess whether other neuropathologic changes, such as loss of cortical neurons and concomitant AD pathology, correlate with LB formation or significantly contribute to dementia in DLB.

Methods. *Patient selection.* We studied 134 individuals—81 with AD, 20 with DLB, and 33 controls. The 81 cases of AD had been examined and followed in the clinical units of the Massachusetts General Hospital Alzheimer's Disease Research Center and had a subsequent neuropathologic diagnosis of definite AD^{21,22} without evidence of strokes, LB, or other lesions. In 50 cases, tissue was available for detailed quantitative neuropathologic assessments. Published data on 34 of these cases²³ are presented here for comparison. The 20 cases of DLB came from the clinical units of the Massachusetts General Hospital where they had undergone neurologic examinations. In all, the neuropathologic assessment had shown the presence of numerous cortical LB with or without concomitant AD changes. Following the Consortium on DLB guidelines, hematoxylin-eosin sections containing the entorhinal (BA28), cingulate (BA24), temporal (BA21), frontal (BA8/9), and parietal (BA40) regions were scored for the number of LB according to a scale where 0 indicates no LB, 1 indicates less than or equal to five LB, and 2 indicates more than five LB.²⁰ All 20 cases met criteria for neocortical DLB. In addition, 13 met criteria for definite AD (DLB/AD), whereas in the remaining 7 neither a significant number of neuritic plaques²² nor neocortical NFT²⁴ were noted. These seven cases are referred to here as DLB.

All AD, DLB/AD, and DLB cases had clinical histories of dementia with well-documented duration of disease. Most patients had been diagnosed by the clinicians as hav-

ing probable or possible AD. Two of the pathologically confirmed AD/DLB cases had been clinically diagnosed as having PD with dementia on initial presentation. Most of the patients had undergone biannual serial evaluations that included standardized general medical and neurologic examinations as well as neuropsychological assessments over an average of 34.5 ± 19.3 months for the AD group, 17.2 ± 12.1 months for the DLB/AD group, and 26.4 ± 22.4 months for the DLB group. We charted measures of global cognitive and functional impairment derived from the information, memory, and concentration subtest of the Blessed Dementia Scale (IMC-BDS)²⁵ and the Activities of Daily Living (ADL) scale.²⁶ The rate of global cognitive deterioration was calculated as the total change in scores between the first and last test divided by the time elapsed between them.²⁷

The sex distribution, age at death, age at onset, and duration of illness for each subgroup are shown in table 1. No significant differences were observed in age at onset or duration of clinical symptoms among the three dementia groups—AD, DLB/AD, and DLB.

The control tissue for the quantitative histologic assessment came from 33 individuals with normal brains by neuropathologic examination. Data on 14 have been previously reported and are presented here for comparison.²³ None of the 33 control cases met neuropathologic criteria for AD or DLB.²¹ Chart review of these individuals did not reveal evidence for neurologic illness.

Tissue processing. All brains (50 AD, 13 DLB/AD, 7 DLB, and 33 controls) were fixed in 10% buffered formalin or 4% paraformaldehyde within 36 hours after death. With a freezing sledge microtome, 50-µm-thick sections were obtained from blocks containing the superior temporal sulcus (STS) region. Adjacent sections were stained using the Nissl method for neuronal counts and immunohistochemistry with antibodies against ubiquitin (Chemicon, Temecula, CA) to visualize LB, paired helical filaments (PHF-1, courtesy of Dr. Peter Davies, Bronx, NY) for NFT, and β-amyloid (10D5, courtesy of Drs. Peter Seubert and Dale Schenk, Athena Neurosciences, South San Francisco, CA) for SP.

Quantitation of neurons, LB, NFT, and SP. Neuronal, LB, and NFT counts were performed following the stereologic optical disector procedure as previously described.²³ In brief, the region counted was located in the inferior bank of the STS, approximately 1 cm medial to the crown of the gyrus. Volume density was assessed in an area measuring 700 µm along the pial surface by the full width of the gray matter. Data were recorded by the Bioquant Im-

age Analysis System (Nashville, TN). The total number of STS neurons, LB, and NFT per section was estimated for each case by multiplying the volume density obtained from the optical disector counts by the volume of the STS measured on each cross-section. An estimate of neuronal loss in AD, DLB/AD, and DLB brains was calculated by subtracting the number of neurons in each individual case from the average number obtained in the control group. LB and NFT counts were obtained from adjacent sections using anti-ubiquitin and PHF-1 antibodies, respectively. In two cases where numerous LB coexisted with abundant NFT in the STS, a double fluorescent labeling was performed in the same section to ensure the accuracy of LB counts and to distinguish between small, globose, ubiquitin-positive NFT and LB.

The percentage of cortical area covered by SP (amyloid burden) was assessed in the same brain region using an anti-A β monoclonal antibody (10D5). The video images were captured, and an optical density threshold able to discriminate the immunostaining was obtained. In each field manual editing eliminated artifacts and the staining associated with blood vessels.^{23,28}

Statistical analysis. The comparison of BDS and ADL scale rates between AD and DLB and DLB/AD groups was done by a multivariate analysis that included initial BDS and ADL scale scores and the total length of follow-up as possible predictors of global clinical decline. A linear regression analysis was used to compare neuron number in STS among AD, DLB/AD, and DLB groups and to correlate neuron number with duration of illness, LB, NFT, and SP. The comparison of total number of neurons between AD, DLB/AD, and DLB versus control group; the amyloid burden between AD, DLB/AD, and DLB groups; and the number of NFT between AD and DLB/AD groups was performed by analysis of variance, with statistical significance at the $p < 0.05$ level. The comparison of the adjusted means of number of neurons between AD and DLB/AD groups was done by analysis of covariance (ANCOVA) with statistical significance at the $p < 0.05$ level.

Results. *Goal 1: Clinicopathologic correlation studies of DLB.* We compared the rate of clinical decline in these neuropathologically defined diagnostic groups. Serial scores in the IMC-BDS were charted to assess cognitive change, and serial scores in the ADL scale were used to assess functional decline. No significant differences among AD, DLB/AD, and DLB groups were observed in the IMC-BDS and ADL scale scores at entry (table 2). Thus, the groups were well matched for level of cognitive impairment at entry. No significant differences were detected in the annual rate of change in IMC-BDS score among the three

Table 2 Rates of cognitive and functional decline

Scale	AD (n = 53)	DLB/AD (n = 8)	DLB (n = 4)
1st BDS	15.9 \pm 9.1	17.4 \pm 10.8	10.2 \pm 6.8
1st ADL	49.5 \pm 24.6	41.4 \pm 5.9	49.5 \pm 16.6
BDS rate, points/y	4.5 \pm 4	5.3 \pm 6.3	4.5 \pm 5.3
ADL rate, points/y	9.1 \pm 10	43.2 \pm 39.5	28.5 \pm 14.2
Follow-up, mo	34.5 \pm 19.3	17.2 \pm 12.1	26.4 \pm 22.4

Values are mean \pm SD.

DLB = dementia with Lewy bodies; BDS = Blessed Dementia Scale; ADL = Activities of Daily Living Scale.

groups. However, DLB/AD and DLB cases had higher rates of annual decline in the ADL scale (43.2 \pm 39.5, $p < 0.01$, and 28.5 \pm 14.2, $p = 0.05$, respectively) when compared with the AD group (9.1 \pm 10). A multivariate analysis looking at BDS/ADL scale rates, covarying first BDS/ADL scale score and time interval between first and last BDS/ADL scale score, confirmed that there was no significant difference in the annual BDS score rates of decline between AD and DLB/AD and DLB groups. However, we still found a marginal difference for annual ADL scale score rates of decline ($p = 0.08$), with higher rates in DLB/AD and DLB than in AD patients, probably reflecting the increased behavioral and motor deficits in the DLB groups.

We evaluated standardized clinical records using the new Consortium on DLB clinicopathologic criteria in this series. The Consortium on DLB has proposed the following as cardinal clinical features that predict DLB with high likelihood: the presence of recurrent visual hallucinations (well formed and detailed), spontaneous motor features of parkinsonism, and fluctuating cognition²⁰ (table 3). Reliable data were available from the charts on the presence of recurrent visual hallucinations and spontaneous parkinsonism because these data are explicitly recorded in the standardized clinical examination forms that are completed at every visit to the Memory Disorder Unit of the Massachusetts Alzheimer's Disease Research Center. Data on fluctuating cognition were not collected in a uniform way, and therefore they are not considered further in our analysis. Based on the information available, at the time of the first clinical evaluation a diagnosis of possible or probable DLB could have been made only in 53% of the neuropathologically confirmed DLB/AD and DLB cases. However, this increased to 90% at later points of the clinical course (see table 3). In addition, 17% of the neuropatho-

Table 3 Clinical features and number (%) of autopsy-confirmed DLB patients meeting consensus criteria for possible or probable DLB at clinical examination

Timepoint	Dementia	Visual hallucinations	EPS	Possible DLB ²⁰	Probable DLB ²⁰	Total
First visit	10/13 (77%)	3/13 (23%)	9/13 (69%)	5/13 (38%)	2/13 (15%)	7/13 (53%)
Course	13/13 (100%)	8/11 (73%)	8/11 (73%)	4/11 (36%)	6/11 (54%)	10/11 (90%)

The patients met consensus criteria in the absence of documentation of fluctuating cognition. In 2 of 13 patients, clinical data on recurrent visual hallucinations and spontaneous motor features of parkinsonism were only available at the time of the initial visit.

DLB = dementia with Lewy bodies; EPS = extrapyramidal signs.

Table 4 Quantitative neuropathologic studies

Study	AD (n = 50)	DLB/AD (n = 13)	DLB (n = 7)	Controls (n = 33)
No. LB per STS section, $\times 10^3$	—	1.57 \pm 1.36	0.79 \pm 0.53	—
No. NFT per STS section, $\times 10^3$	6.98 \pm 3.95	4.12 \pm 4.08	0.067 \pm 0.091	—
Amyloid burden, %	7.66 \pm 3.02	8.87 \pm 3.13	5.60 \pm 3.02	<1
No. neurons per STS section, $\times 10^3$	48.2 \pm 22.6*	56.2 \pm 17.7*	82.8 \pm 21.7	92.9 \pm 9.1

Values are mean \pm SD.

* $p < 0.001$. Number of neurons in the STS per 50- μ m-thick section in AD and DLB/AD brains vs controls.

DLB = dementia with Lewy bodies; STS = superior temporal sulcus; NFT = neurofibrillary tangles.

logically confirmed pure AD cases met Consortium on DLB criteria for possible DLB and 0% for probable DLB at entry. This increased to 30% for possible DLB and 2% for probable DLB during the clinical course despite the absence of LB at autopsy.

Goal 2: Relation between LB number and duration of illness. All DLB/AD and DLB cases presented in this series met pathologic Consortium on DLB criteria for the neocortical DLB category. For the purpose of detailed neuroquantitative assessments, the STS region was selected for several reasons: 1) It contains one of the brain areas designated by the Consortium on DLB for evaluation of LB distribution and frequency (BA21). 2) It represents a high order association area that receives multiple inputs from association and limbic cortical regions. 3) It is consistently affected by LB in the neocortical category of DLB, and by SP and NFT in AD. 4) Previously studied control and AD cases are available for comparison.

Data from the quantitative neuropathologic assessments are summarized in table 4. We found no correlation between number of LB in the STS and duration of clinical dementia symptoms in either the DLB or DLB/AD groups ($R^2 = 0.16$, $p = 0.16$, not significant [NS]). Whether the number of LB might correlate with other clinical measurements of dementia severity could not be reliably addressed in this study due to the lack of enough cases with a BDS score within a year from death.

Goal 3: Correlation between LB and other cortical pathologic changes. We evaluated the possibility that neuropathologic changes other than neocortical LB accumulation, such as neuronal loss or AD-related pathology (NFT and SP), may significantly contribute to the clinical progression of dementia in DLB and DLB/AD. We have previously reported a dramatic neuronal loss in the STS of pure AD brains that parallels the chronologic evolution of dementia.²³ In the current series, the average number of STS neurons per 50- μ m-thick section in the control group was $92,900 \pm 9,100$ (see table 4). No significant gender differences were detected in the number of STS neurons in control brains. The total number of STS neurons was reduced by 49% in pure AD ($p < 0.001$) and by 40% in DLB/AD brains ($p < 0.001$) compared with the average number of neurons estimated in the control group. Of note, a smaller amount of neuronal loss was detected in the DLB group where an average of only 11% of STS neurons were lost compared with control brains ($p = 0.21$, NS) (figure 1). This finding points to a remarkably better preservation of neuronal number in the STS association cortex in DLB

brains compared with pure AD or DLB/AD brains. We tested whether AD and DLB/AD groups differed significantly in the number of neurons in the STS after covarying for duration of illness. There was a within-group relation between the number of neurons and the logarithm of duration of illness ($p < 0.05$). After an ANCOVA adjustment was made for this curvilinear relationship, the DLB/AD group had a higher adjusted mean number of neurons in the STS than the AD group ($p < 0.02$). Thus, the amount of neuronal loss in the STS was greater in AD than in DLB/AD brains after taking into account duration of illness (figure 2). In addition, the number of LB did not correlate with STS neuronal loss in either DLB/AD or DLB brains.

Finally, we evaluated the possible contribution of concomitant AD-type pathologic changes in the association cortices of DLB brains. Of note, assessment of SP showed abundant and comparable amounts of A β deposits in the

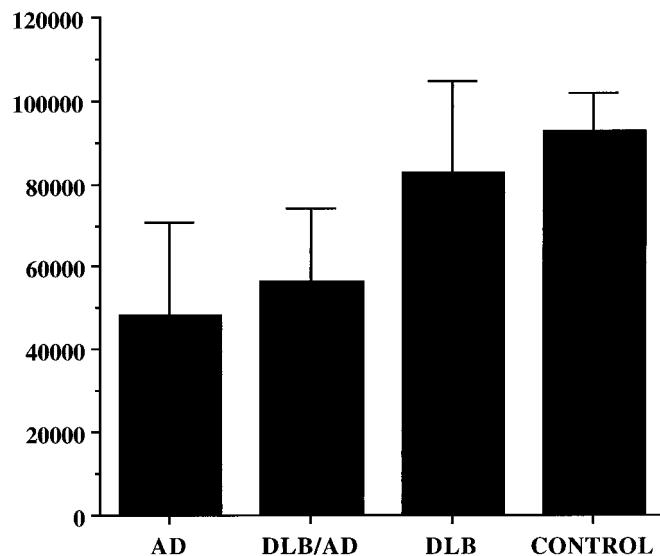


Figure 1. The average total number of neurons in the superior temporal sulcus (STS) volume assessed was reduced by 49% in the AD group ($n = 50$) ($p < 0.001$), by 40% in the dementia with Lewy bodies (DLB)/AD group ($n = 11$) ($p < 0.001$), and by 11% in the DLB group ($n = 6$) ($p = 0.21$, not significant) when compared with the control group ($n = 33$). Data were not available on two DLB/AD cases and one DLB case. y Axis = number of STS neurons per 50- μ m-thick section.

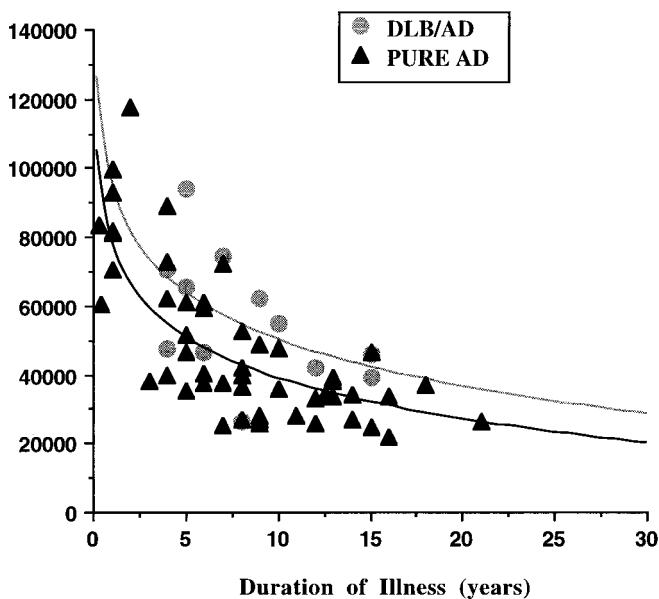


Figure 2. There was a within-group relation between the number of neurons and the logarithm of duration of illness ($p < 0.05$). After an analysis of covariance adjustment was made for this curvilinear relationship, the dementia with Lewy bodies (DLB)/AD group had a higher adjusted mean number of neurons in the superior temporal sulcus (STS) than the AD group ($p < 0.02$). *y* Axis = number of STS neurons per 50- μ m-thick section.

STS of all AD, DLB/AD, and DLB groups with the exception of one brain from the latter group (see table 4). However, none of the DLB cases (by definition) had enough neuritic plaques, (despite abundant A β diffuse deposits) to make the diagnosis of concomitant AD according to CERAD criteria.²² The percentage of STS covered by A β (amyloid burden) did not correlate with duration of illness or amount of neuronal loss in any of the groups. The number of NFT in DLB/AD brains was lower than it was in pure AD brains ($p < 0.05$) despite similar duration and degree of cognitive impairment, and did not correlate with any of the above variables. Furthermore, the presence of LB did not correlate positively with the amount of A β in either the DLB/AD or DLB groups ($R^2 = 0.04$, NS) or the number of NFT in the DLB/AD group ($R^2 = 0.11$, NS).

Discussion. This study suggests the following: 1) The new Consortium on DLB criteria are useful in clinicopathologic studies on DLB, but their sensitivity and specificity at the time of the first clinical evaluation are moderate. 2) In pathologically confirmed DLB and DLB/AD brains, LB formation in association cortices does not significantly increase with time or the progression of other neuropathologic changes, e.g., neuronal loss and concomitant AD-type pathology. 3) Unlike pure AD or DLB/AD, there is minimal neuronal loss in the STS of DLB brains without concomitant neuritic SP or NFT.

Our study focuses on two aspects of DLB—the clinical presentation of autopsy-confirmed cases and a quantitative neuropathologic study of the disease in comparison with AD. With regard to the clinical

presentation, we applied the Consortium on DLB guidelines to further assess the clinicopathologic correlations of DLB with and without concomitant AD neuritic pathology.²⁰ In this study patients were divided into three pathologic groups based on the presence or absence of LB, neuritic plaques, and NFT—pure AD, DLB/AD, and DLB. None of the pure AD cases met pathologic Consortium criteria for DLB. All DLB/AD and DLB cases met Consortium criteria for DLB, falling into the category of neocortical DLB. The sensitivity and specificity of the Consortium clinical criteria in this series for dementia, hallucinations, and parkinsonism are 53% and 83%, respectively, at the patient's initial visit and 90% and 68%, respectively, if data from all clinic visits are considered.

The three dementia groups studied here had similar mean age at dementia onset and length of survival from the onset of dementia symptoms. Furthermore, AD, DLB/AD, and DLB patients had similar degrees of global cognitive decline and functional impairment at entry as measured by the IMC-BDS and the ADL scale. Even though the annual rates of cognitive decline, as measured by IMC-BDS serial scores, were comparable among the three groups, we noticed a faster decline in ADL performance in DLB/AD and DLB patients than in AD patients. We believe this observation might reflect the additional burden that recurrent visual hallucinations and extrapyramidal symptoms adds in DLB cases to the successful performance of routine daily activities evaluated by this scale.

We also applied quantitative neuropathologic techniques to DLB brains because the neuropathologic substrate of dementia in DLB is uncertain. Whether LB are true markers of neuronal injury, represent a protective cell response, or are simply a nonspecific epiphenomenon is unknown. Furthermore, the mechanism by which cortical LB formation may lead to a clinical dementia syndrome, the amount required to produce a clinically detectable cognitive impairment, and their correlation with the progression of symptoms and with AD concurrent pathologic changes are controversial.^{19,29-31} Some neuropathologic studies have suggested that the cortical LB burden is a meaningful measure that correlates with the severity of cognitive impairment before death.^{19,29,31} No significant correlation was observed between the number of LB in the STS and the duration of the clinical symptoms of dementia in either DLB/AD or DLB brains in this series, although we were unable to compare directly the number of LB with neuropsychological testing because, among the DLB cases, few had been tested within 1 year of death.

Based on these results, we suggest that other pathologic changes including loss of neurons or synapses or AD-related pathology might be more directly related to dementia in DLB than LB formation. When compared with nondemented controls, almost one-half the neuronal population nor-

mally present in the STS region is lost in an average AD brain.²³ The data from this study indicate a similar behavior in DLB/AD brains with 40% of STS neuronal loss as an average, even though the amount of neuronal loss in AD brains is significantly greater than it is in DLB/AD after adjusting for duration of illness. However, the preservation of overall STS neuron number in pure DLB brains without concomitant AD pathology suggests that dementia in DLB is not due to a generalized neuronal depletion in areas affected by LB.

Thus, if not neuronal loss, what underlies dementia in DLB? Several alternatives can be suggested. LB might affect and destroy a relatively small but critical subpopulation of cortical or subcortical neurons leading to widespread functional impairments. Alternatively, LB might lead to functional or anatomic synaptic disruption. Some studies have suggested that in DLB the amount of synapse loss might be comparable with that seen in pure AD brains,¹⁷ whereas others have failed to show any significant correlation between anti-synaptophysin reactivity and degree of cognitive impairment in DLB.¹⁹ Nevertheless, the possibility of an underlying synaptic alteration in DLB has very recently gained increased attention due to the finding of mutations in the gene that codifies for the presynaptic protein α -synuclein in families with autosomal dominant PD.^{32,33}

Finally, we have evaluated the relationship between DLB and AD. All the DLB/AD and DLB brains examined in this study but one had amounts of A β deposits in the STS comparable with those seen in pure AD. These results are in agreement with previous reports.^{34,35} No correlation was found, however, between the amount of amyloid deposited in the STS and LB counts. Could it be assumed that amyloid deposition is directly related to dementia in DLB? Several observations argue against this possibility. It is known that some elderly individuals have abundant diffuse amyloid deposits in the cortex without apparent cognitive deficits.³⁶⁻³⁸ Furthermore, clinicopathologic studies have demonstrated that diffuse plaques have little or no correlation with clinical measures of cognition^{39,40} and are not accompanied by synaptic loss.⁴¹

Even though DLB/AD brains had abundant NFT in the STS coexisting with LB and amyloid deposits, their number was significantly lower than it was in AD despite similar duration and severity of dementia. This finding is in agreement with studies reported by others.^{3,42,43} Furthermore, NFT number did not correlate with the number of LB. Taken altogether, the lack of an obvious positive relationship of LB formation to the amount of Alzheimer-type changes (neuritic SP, NFT, neuronal loss) observed in this series favors a model where DLB and AD likely represent two distinct pathologic processes, even though their frequent co-occurrence raises the possibility of a common underlying risk factor.

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Metrifonate treatment of AD

Influence of *APOE* genotype

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Article abstract—*Objective:* To investigate whether an interaction exists between *APOE* genotype and the response of AD patients to metrifonate treatment and whether *APOE* genotype independently affects the rate of AD progression. *Background:* Metrifonate is a new acetylcholinesterase inhibitor for the treatment of AD symptoms. *Methods:* Data were pooled from four prospective, randomized, double-blind, placebo-controlled clinical trials and analyzed retrospectively. A total of 959 patients who received once-daily placebo ($n = 374$) or metrifonate (30 to 60 mg based on weight or a 50-mg fixed dose, $n = 585$) for up to 26 weeks agreed to *APOE* genotyping. *Results:* Metrifonate clearly improved the cognitive performance of the AD patients when compared with placebo (Alzheimer's Disease Assessment Scale—Cognitive Subscale [ADAS-Cog], $p = 0.0001$). The interaction of *APOE* genotype and the metrifonate effect on cognitive performance were not significant ($p = 0.25$). Metrifonate also clearly improved the global function of the AD patients when compared with placebo (Clinician's Interview-Based Impression of Change with Caregiver Input [CIBIC-Plus], $p = 0.0001$). The interaction of *APOE* genotype with the metrifonate effect on global function also was not significant ($p = 0.70$). No significant three-way interactions were observed among *APOE* genotype, gender, and response to metrifonate treatment (ADAS-Cog, $p = 0.68$; CIBIC-Plus, $p = 0.26$). *APOE* genotype did not influence disease progression as evaluated by either cognitive performance (ADAS-Cog, $p = 0.93$) or global function (CIBIC-Plus, $p = 0.64$). *Conclusions:* The findings from these studies of up to 26 weeks' duration do not clearly support an interaction between *APOE* genotype and metrifonate treatment effects. They suggest that *APOE* genotypes do not necessarily predict an AD patient's response to metrifonate treatment and that *APOE* genotype may not influence the rate of disease progression for patients with mild to moderate AD. **Key words:** Acetylcholinesterase inhibitor—Dementia—*APOE* genotype—Clinical trial—Cognition—Global function.

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Alzheimer's disease (AD), a progressive neurodegenerative disorder, is characterized neuropathologically by the presence of extracellular neuritic plaques and intracellular neurofibrillary tangles that are accompanied by a selective and severe loss of central cholinergic neurons.¹ Several factors have been proposed as influencing the risk of patients for developing AD, including *APOE* genotype.² ApoE redistributes lipids among cells for different organs, including the brain.³ It has been implicated in the repair of damaged neuronal membranes and plasticity and hence may play a role in the response to neuronal injury or trauma. The gene for apoE is localized to human chromosome 19 and exists in three allelic forms, designated *APOE2*, *APOE3*, and *APOE4*.⁴ Although the homozygous *APOE3* genotype is the most common, the presence of the *APOE4* allele has been associated with both sporadic and familial late-onset types of AD.⁵ Moreover, the presence of the homozygous *APOE4* genotype has been associated with disease onset in the 60s for as many as 50% of individuals carrying this genotype.⁶ One recent estimate indicates that approximately 60 to 70% of all AD cases are associated with the *APOE4* genotype.⁷

In the brains of AD patients, *APOE* genotype may

correlate with certain neuropathologic features of the disease. Several investigators have reported a significant correlation between increasing *APOE4* allele copy number and increased cortical neuritic plaque density,^{8–11} although this finding has not been confirmed in all studies.^{12,13} *APOE4* allele copy number has been related inconsistently to the appearance of neurofibrillary tangles, with some studies showing a positive correlation^{8,11,14} and others no correlation.^{9,10,12} *APOE4* allele copy number also has been shown to have an inverse relationship with several biochemical markers of cholinergic activity in the brain and CSF of AD patients, suggesting that AD patients carrying an *APOE4* allele have a more severe cholinergic deficit than those who do not.^{8,15–18} The association of the *APOE4* genotype with diverse pathologic and biochemical abnormalities would appear to support an effect of *APOE* genotype on disease progression.

Although *APOE4* genotype appears to modify the neuropathology of AD, it is currently unclear whether this genotype influences the clinical course of the disease. Evaluations of the cognitive performance of AD patients over the course of the disease suggest no effect of *APOE* genotype on disease

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progression.¹⁹⁻²¹ However, retrospective analyses of clinical studies with tacrine, an inhibitor of the acetylcholine-metabolizing enzyme acetylcholinesterase (AChE), suggest that the *APOE* genotype of an AD patient may influence the patient's response to treatment with cholinesterase inhibitors.^{22,23}

Metrifonate is a new AChE inhibitor developed for the treatment of AD.^{24,25} In previous studies, metrifonate significantly improved the cognition and global function of patients with mild to moderate AD.²⁶⁻³⁰

The objective of the current retrospective analysis was to use treatment data pooled from four large metrifonate trials^{16-28,30} to investigate whether an interaction exists between treatment effect and *APOE* genotype, and whether *APOE* genotype independently affects the rate of disease progression.

Methods. *Study design.* The four studies for this pooled analysis were prospective, multicenter, randomized, double-blind, parallel-group, placebo-controlled trials of metrifonate in patients with probable AD of mild to moderate severity.

Each of the four clinical trials selected for the pooled analyses met the Food and Drug Administration guidelines for establishing efficacy of an AD therapeutic agent. In this regard, each trial was at least 12 weeks in duration and evaluated the efficacy of metrifonate in improving cognitive performance using the Alzheimer's Disease Assessment Scale—Cognitive Subscale (ADAS-Cog)³¹ and global functional status using the Clinician's Interview-Based Impression of Change with Caregiver Input (CIBIC-Plus).³²

The studies were conducted in accordance with Institutional Review Board guidelines at the participating centers. The studies were fully explained to all patients and their caregivers, and informed consent for participation was given by the patient as well as the legal representative or family caregiver.

Patient selection. Patient populations were selected using similar inclusion and exclusion criteria for each of the four trials.

Inclusion criteria. Patients were evaluated by clinical interview, psychiatric assessment, physical and neurologic examinations, and laboratory studies. All participants met the diagnosis for probable AD according to criteria of the *Diagnostic and Statistical Manual of Mental Disorders, 4th Edition* (DSM-IV)³³ or the National Institute of Neurological and Communicative Disorders and Stroke—Alzheimer's Disease and Related Disorders Association.³⁴ Patients had Mini-Mental State Examination (MMSE)³⁵ scores between 10 and 26, modified Ischemia Scale³⁶ scores of less than 4, and weighed between 43 and 98 kg. All participating patients had caregivers with whom they were in contact at least four times a week.

Exclusion criteria. Patients were excluded from a study if they had a dementia other than probable AD. Patients underwent CT or MRI to exclude vascular dementia, hydrocephalus, and intracranial mass lesions. Patients with cognitive impairment attributable to toxic or alcoholic causes or to head trauma were excluded. Patients also were prevented from study participation if they had a history of medical disorders associated with dementia not at-

tributable to AD. Patients were excluded if they had clinically significant medical problems, including cancer, within the preceding 5 years, poorly controlled diabetes, asthma or chronic obstructive pulmonary disease, gastrointestinal obstruction, or clinically significant hepatic, renal, cardiac, or pulmonary insufficiency. Lastly, patients were excluded from the study if they had taken metrifonate in the past for the treatment of AD or were taking any of the following medications: potentially cognition-enhancing agents (choline, lecithin, Hydergine, tacrine, donepezil), drugs with significant cholinomimetic or anticholinergic activity, centrally active antihypertensive agents, anticonvulsants, antacids or cimetidine, or any investigational drug (within 30 days of screening). Psychoactive drug use for temporary treatment of behavioral abnormalities was restricted but not completely disallowed.

Metrifonate dosing. A total of 1,757 patients were enrolled in these studies and were randomized to receive either placebo (n = 550) or metrifonate (n = 1,207). Patients in study A²⁶ received for a total of 12 weeks' once-daily administrations of placebo (n = 120) or one of three metrifonate doses based on weight: loading doses of 25 to 45 mg (0.5 mg/kg, n = 121), 45 to 80 mg (0.9 mg/kg, n = 120), or 100 to 180 mg (2.0 mg/kg, n = 119) for 2 weeks followed by maintenance doses of 10 to 20 mg (0.2 mg/kg), 15 to 25 mg (0.3 mg/kg), and 30 to 60 mg (0.65 mg/kg), respectively, for 10 weeks.

Patients in study B²⁷ received once-daily administrations of placebo (n = 135) or metrifonate (n = 273) for 26 weeks. Metrifonate-treated patients received a loading dose of 100 to 180 mg based on weight (2.0 mg/kg) for 2 weeks and then a maintenance dose of 30 to 60 mg (0.65 mg/kg) for 24 weeks.

Study C³⁰ patients received placebo (n = 208) or participated in one of two metrifonate dosing regimens for a total of 26 weeks. Patients in both metrifonate groups received a once-daily loading dose of 80 mg or 120 mg based on weight <65 kg or ≥65 kg, respectively, for 2 weeks. This was followed by the administration of a once-daily maintenance dose of 40 mg or 50 mg based on weight <65 kg or ≥65 kg, respectively, for 24 weeks (n = 200), or of a once-daily maintenance dose of 60 mg or 80 mg based on weight <65 kg or ≥65 kg, respectively, for 24 weeks (n = 197).

Patients in study D²⁸ received either placebo (n = 87) or metrifonate 50 mg (n = 177) once daily for 26 weeks. Study D did not use a loading dose regimen.

Efficacy measures. The primary efficacy variables in all four studies were the ADAS-Cog and the CIBIC-Plus. The ADAS-Cog is a validated, multi-item instrument that examines various aspects of cognitive ability, including memory, orientation, attention, and reasoning. The scores range from 0 to 70, with higher scores reflecting greater cognitive impairment.

The CIBIC-Plus assesses global function. It is not a single or standardized instrument. The CIBIC-Plus used in metrifonate trials represents the evaluation by a skilled clinician based on observation of the patient and an interview with the patient caregiver. The interview may have considered the following aspects of patient function: global, cognitive, behavioral, and activities of daily living. The CIBIC-Plus comprises a seven-point scale, with a score of 1 indicative of marked improvement, a score of 7 indicative of marked worsening, and a score of 4 indicative of no

Table 1 Demographic characteristics of patients at baseline

Characteristic	Placebo (n = 374)			Metrifonate (n = 585)		
	0 APOE4 (n = 132)	1 APOE4 (n = 183)	2 APOE4 (n = 59)	0 APOE4 (n = 236)	1 APOE4 (n = 281)	2 APOE4 (n = 68)
	Age, y	73.2	74.1	70.5	73.5	74.4
Weight, kg	69.6	66.5	67.7	67.1	65.5	68.3
Height, cm	164	163	164	164	163	166
Gender, % male	42.4	32.8	37.3	38.6	32.7	42.6
Race, % white	73.5	72.1	69.5	78.8	80.4	77.9
Education						
No schooling, %	0	2.2	0	0	0.7	1.5
Grammar school/equivalent, %	31.1	38.8	23.7	26.7	27.8	25.0
High school/equivalent, %	44.7	35.0	42.4	44.5	48.8	42.6
College/equivalent, %	24.2	24.0	33.9	28.0	22.8	30.9
ADAS-Cog score, mean	22.0	22.7	21.3	22.8	22.9	25.4
MMSE score, mean	19.3	18.5	19.4	18.8	18.5	18.2

ADAS-Cog = Alzheimer's Disease Assessment Scale—Cognitive Subscale; MMSE = Mini-Mental State Examination.

change. Evaluation and scoring for the CIBIC-Plus was left to the discretion and expertise of the clinician. Anchor points were not defined in an effort not to artificially increase the sensitivity of the scale. Additionally, evaluations using the CIBIC-Plus and the ADAS-Cog were conducted by independent raters who had no knowledge of the score received on the other outcome measure.

APOE genotyping. *APOE* genotyping was performed as described by Hixson and Vernier.³⁷ In brief, restriction enzyme isoform genotyping used oligonucleotides to amplify *APOE* gene sequences containing amino acid positions 112 and 158. The amplification products were digested with *Hha*I and electrophoresed on polyacrylamide gels. Each isoform was characterized by a unique combination of *Hha*I fragment sizes. Evaluation of the fragments permitted the identification of all homozygote and heterozygote genotype combinations.

Data analyses. In the pooled analyses, patients receiving a metrifonate maintenance dose of 30 to 60 mg based on weight (studies A and B), 40 mg or 50 mg based on weight (study C), or a 50-mg fixed dose (study D) were considered to be the same treatment group (n = 769). Note that patients from study A who received one of the two lower doses of metrifonate (i.e., <30 to 60 mg/d based on weight, n = 241) were not included in the pooled analysis because these doses were too low to yield consistent clinical benefits. Because the patient group receiving a maintenance dose of 60 or 80 mg (n = 197) was included in only one of the four studies (study C), because it may have generated a greater treatment response than the 30- to 60-mg dose (as suggested by other analyses), and because its division according to *APOE* genotype resulted in analytically prohibitive sample sizes (in some cases, n < 10), it too was excluded from the pooled analysis. Placebo-treated patients pooled from all four studies comprised the placebo group (n = 550).

Patient validity was as determined for the individual studies. Of the 1,319 patients randomized to the treatment

groups under consideration, all were included in the intent-to-treat (ITT) analysis, with the exception of those 23 who did not take any study medication or did not have a post-baseline efficacy assessment (placebo, n = 9; metrifonate, n = 14), or both. The method of handling assessments missing due to premature study discontinuation (placebo, n = 58; metrifonate, n = 115) involved last observation carried forward.

Both efficacy variables were analyzed in two ways: 1) as a change from baseline and 2) as a drug-versus-placebo difference (i.e., treatment difference) in the change from baseline score. The primary analysis was of change from baseline at treatment end point (data from week 12 for study A and week 26 for studies B to D analyzed collectively).

Analyses were stratified by study. Treatment-by-number of alleles interactions were determined from analysis of variance (ANOVA) models with effects for study, drug, number of alleles, and drug-by-number of alleles interaction. For each "number of alleles" subgroup, ANOVA models with effects for study and drug were used. An evaluation of drug-by-study-by-number of alleles subgroup means suggested that results were similar across studies.

Results. Patient population. Of the pooled metrifonate and the pooled placebo patients valid for the ITT analysis (n = 1,319), 959 agreed to *APOE* genotyping. The demographic characteristics of the studied patients are summarized in table 1. The placebo and metrifonate treatment groups that were defined based on *APOE4* allele copy number were characterized by a similar mean age, weight, and height, as well as similar distributions according to race, gender, and educational level. All six groups also had comparable mean ADAS-Cog and MMSE scores at baseline, suggesting that their degree of cognitive impairment was similar.

Demographic characteristics of patients who agreed to *APOE* genotyping were compared statistically with these characteristics of patients who did not agree to genotyping

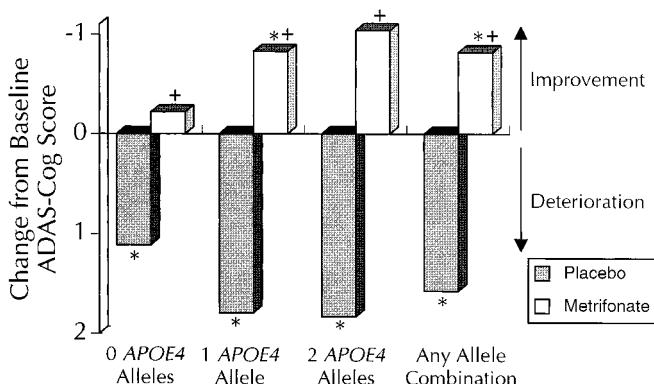


Figure 1. Effect of APOE genotype on the cognitive response of AD patients to metrifonate treatment. Abscissa: APOE genotype. Ordinate: Change from baseline Alzheimer's Disease Assessment Scale-Cognitive Subscale (ADAS-Cog) score at study end point. (Study end point reflects week 12 for study A and week 26 for studies B, C, and D.) Patients analyzed were those in the intent-to-treat population (last observation carried forward, $n = 959$). * $p < 0.05$ when compared with baseline. † $p < 0.05$ when compared with placebo.

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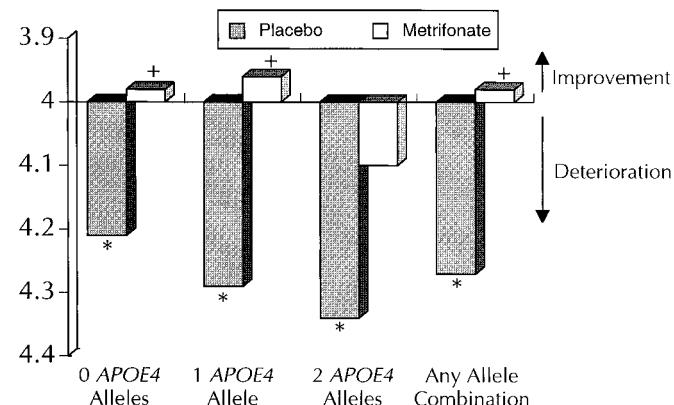


Figure 2. Effect of APOE genotype on the functional response of AD patients to metrifonate treatment. Abscissa: APOE genotype. Ordinate: Clinician's Interview-Based Impression of Change with Caregiver Input (CIBIC-Plus) score at study end point. (Study end point reflects week 12 for study A and week 26 for studies B, C, and D.) Patients analyzed were those in the intent-to-treat population (last observation carried forward, $n = 959$). * $p < 0.05$ when compared with baseline. † $p < 0.05$ when compared with placebo.

($n = 337$). Of those patients who did not agree to genotyping, the majority participated in study C (115/167 placebo-treated patients [69%] and 119/170 metrifonate-treated patients [70%]). Thus, any differences between those patients who did and who did not agree to genotyping tended to be driven by the patients from this one study. In this regard, the only significantly different characteristic ($p < 0.05$) between patients who did and who did not agree to genotyping was gender. Patients who did not agree to genotyping were more likely to be female than those patients who had their genotype determined.

Effect of APOE genotype on response to metrifonate as assessed by cognitive performance. The effect of metrifonate (30 to 60 mg) on the cognitive performance of AD patients at treatment end point, as assessed using the ADAS-Cog, is illustrated in figure 1. Metrifonate significantly improved the cognitive performance of the AD patients when compared with placebo, regardless of APOE genotype. Statistical analysis revealed that the interaction between APOE4 allele copy number and the treatment effect as assessed by the ADAS-Cog score was not significant ($p = 0.25$). However, there was a trend for placebo patients with an APOE4 allele to perform worse than those patients who did not have an APOE4 allele (see figure 1). Moreover, this trend was related to the number of APOE4 alleles. APOE4 homozygotes showed the greatest deterioration in ADAS-Cog score relative to baseline ($\Delta = 1.83$ points, $p = 0.01$), APOE4 heterozygotes had a very similar degree of deterioration ($\Delta = 1.79$ points, $p = 0.0001$), and patients without an APOE4 allele had the least worsening in the ADAS-Cog score ($\Delta = 1.11$ points, $p = 0.02$).

Interestingly, there also was a trend for the metrifonate-treated patients to perform better on the ADAS-Cog with increasing APOE4 allele copy number (see figure 1). Thus, patients treated with metrifonate who were homozygous for APOE4 showed the greatest improvement relative to baseline ADAS-Cog score ($\Delta = 1.04$ points, $p = 0.16$), patients heterozygous for APOE4 manifested an

intermediate level of improvement ($\Delta = 0.83$ points, $p = 0.02$), and patients who did not carry an APOE4 allele had the least-improved ADAS-Cog score ($\Delta = 0.22$ points, $p = 0.57$). Similarly, at treatment end point, the drug-versus-placebo differences for the change from baseline ADAS-Cog scores were larger with increasing APOE4 allele copy number (two alleles, $\Delta = 2.87$, $p = 0.005$; one allele, $\Delta = 2.61$, $p = 0.0001$; 0 alleles, $\Delta = 1.33$, $p = 0.03$).

The interaction between the metrifonate treatment effect as assessed by ADAS-Cog score and APOE genotype also was analyzed when APOE genotype was dichotomized (i.e., 0 versus one and two alleles). This analysis revealed that a trend for an interaction between treatment effect and genotype did exist, although it was not statistically significant ($p = 0.11$).

Effect of APOE genotype on response to metrifonate as assessed by global function. The effect of metrifonate (30 to 60 mg) on the global function of the AD patients, as evaluated by the CIBIC-Plus score at treatment end point, is shown in figure 2. Metrifonate improved the global function of the AD patients when compared with placebo. However, the interaction of APOE genotype and the metrifonate effect on global function, as evaluated by the CIBIC-Plus, also was not significant ($p = 0.70$). However, a trend again was observed for the placebo patients for a deterioration in global function based on the APOE4 allele dose (see figure 2). Placebo patients carrying two APOE4 alleles manifested the most profound deterioration in the CIBIC-Plus score ($\Delta = 0.34$ points, $p = 0.003$), patients carrying a single APOE4 allele showed an intermediate deterioration ($\Delta = 0.29$ points, $p = 0.0001$), and patients without an APOE4 allele had the least decline in score ($\Delta = 0.21$ points, $p = 0.01$).

No clear pattern of treatment effect magnitude versus APOE4 allele copy number was observed for the metrifonate-treated patients in regard to global function (see figure 2). Patients treated with metrifonate who possessed a double complement of APOE4 alleles had the

Table 2 Progression of ADAS-Cog and CIBIC-Plus over time for placebo patients

Outcome	No. (0, 1, 2) alleles	Time period	Number of E4 alleles			<i>p</i> Value
			0	1	2	
ADAS-Cog	132, 182, 59	12 weeks	0.70	0.55	1.12	0.73
	107, 146, 44	18 weeks	1.45	1.78	1.02	0.64
	107, 146, 44	26 weeks	1.37	2.47	2.00	0.30
CIBIC-Plus	130, 183, 59	12 weeks	4.14	4.06	4.10	0.66
	106, 146, 44	18 weeks	4.24	4.23	4.30	0.88
	106, 146, 44	26 weeks	4.22	4.39	4.32	0.32

Values are mean changes from baseline as estimated by the drug-by-number of alleles interaction model for each outcome variable. Values show a progressive decline in both ADAS-Cog and CIBIC-Plus over the course of 26 weeks with no consistent relationship with the number of E4 alleles. No significant differences were seen at any point in time.

ADAS-Cog = Alzheimer's Disease Assessment Scale—Cognitive Subscale; CIBIC = Clinician's Interview-Based Impression of Change with Caregiver Input.

worst functional status as indicated by a mean 0.10-point deterioration from baseline CIBIC-Plus score ($p = 0.39$). Metrifonate-treated patients with only one *APOE4* allele or no *APOE4* alleles showed comparable levels of function, with a mean 0.04-point ($p = 0.45$) and 0.02-point ($p = 0.77$) improvement from the baseline CIBIC-Plus score, respectively. Moreover, the metrifonate-versus-placebo treatment difference in CIBIC-Plus scores was greatest for metrifonate-treated patients who had only a single *APOE4* allele ($\Delta = 0.34$ points, $p = 0.0001$) and comparably smaller for those metrifonate-treated patients carrying two or no *APOE4* alleles (two alleles, $\Delta = 0.24$, $p = 0.14$; 0 alleles, $\Delta = 0.23$, $p = 0.03$).

The existence of an interaction between the metrifonate treatment effect as assessed by the CIBIC-Plus score and *APOE* genotype also was explored statistically when *APOE* genotype was dichotomized. No such interaction was observed to exist ($p = 0.40$).

Combined effects of APOE genotype and gender on response to metrifonate. A statistical analysis was conducted to determine whether any three-way interactions exist among *APOE* genotype, gender, and response to metrifonate treatment. No three-way interactions were observed among *APOE* genotype, gender, and metrifonate effects on cognitive performance (ADAS-Cog, $p = 0.68$) or global function (CIBIC-Plus, $p = 0.26$).

Effect of APOE genotype on AD progression. To examine the effects of *APOE* genotype on the rate of disease progression, changes from baseline as measured by the ADAS-Cog and CIBIC-Plus were calculated at each point in time over the course of the studies, rather than evaluated at treatment end point, for the placebo patient groups with 0, one, or two *APOE4* alleles. These changes from baseline, together with *p* values for the comparison of means at each time period, are shown in table 2.

For the ADAS-Cog evaluation of cognitive performance, all untreated patients declined progressively over time, regardless of the *APOE* genotype (see table 2). However, those untreated patients with no *APOE4* allele showed the least deterioration at week 26, reflecting a 1.37-point decline from baseline score. Untreated patients carrying a single *APOE4* allele manifested the most deterioration in cognitive performance at week 26, with a 2.47-point deterioration from baseline score. Untreated patients with two

APOE4 alleles had an intermediate level of deterioration in cognitive performance with a 2.00-point worsening from the baseline ADAS-Cog score. There were no significant differences seen between any of the three *APOE* groups at any point in time. The trend suggested that the rate of loss of cognition was not clearly related to the number of *APOE4* alleles.

Similarly, all untreated patients worsened over time in regard to their global function as assessed with the CIBIC-Plus, regardless of their *APOE* genotype. Patients with no *APOE4* allele declined the least in their global function, with a CIBIC-Plus score that was worse by 0.22 points after 26 weeks. Untreated patients carrying one *APOE4* allele manifested the greatest deterioration in global function, with a 0.39-point worsening in the CIBIC-Plus score after 26 weeks. Untreated AD patients with two *APOE4* alleles declined to an intermediate degree, showing a 0.32-point worsening in the CIBIC-Plus score after 26 weeks. There were no significant differences seen between any of the three *APOE* groups at any point in time. As with the ADAS-Cog above, the rate of progression of global function also was not clearly related to the number of *APOE4* alleles.

Discussion. We analyzed retrospectively the interaction between *APOE* genotype and metrifonate effects on cognitive performance and global function of AD patients. Additionally, we examined whether *APOE* genotype independently affects the rate of disease progression as assessed by both cognitive and global function measures. The findings of these analyses, based on studies of up to 26 weeks' duration, only weakly support an interaction between *APOE* genotype and metrifonate treatment effects, but with a statistical value of $p = 0.25$ for the ADAS-Cog and 0.70 for the CIBIC-Plus, this suggests that metrifonate efficacy may not be predicted by *APOE* genotype. Additionally, based on these studies of up to 26 weeks' duration, the clinical course of AD is not influenced by *APOE* genotype. However, the lack of an apparent interaction between *APOE* genotype and metrifonate treatment response, or between *APOE* genotype and disease progression, may reflect a lack

of statistical power to detect such interactions or the relatively short (i.e., 26-week) treatment period evaluated, or both, rather than the absence of such interactions.

Regardless of *APOE* genotype, metrifonate significantly improved the cognitive performance of the AD patients, as evaluated with the ADAS-Cog, and global function, as evaluated with the CIBIC-Plus. Based on previous analyses of tacrine study data,^{22,23} if *APOE* genotype had affected the response to metrifonate treatment, AD patients homozygous for the *APOE4* allele would have been expected to score the worst on both outcome measures. Interestingly, however, metrifonate-treated patients carrying two *APOE4* alleles actually showed the strongest performance of all six groups of AD patients on the ADAS-Cog. Furthermore, although metrifonate-treated patients carrying two *APOE4* alleles had the worst global functional status of the three groups of metrifonate-treated patients, metrifonate-treated patients with a single *APOE4* allele had a mean change in performance at study end point numerically superior to that of those patients with no *APOE4* allele. Thus, *APOE* genotype did not consistently predict any particular pattern of cognitive or global functional response to metrifonate treatment.

In previous analyses of the response of AD patients to tacrine therapy,^{22,23} the effects of *APOE* genotype on the clinical response of AD patients to 30 weeks of tacrine treatment were evaluated retrospectively. A statistically significant interaction was observed between *APOE* genotype and cognitive performance as measured by group mean ADAS-Cog scores; patients phenotyped as carrying one or more *APOE4* alleles had a smaller response to tacrine treatment than patients phenotyped as having no *APOE4* alleles.²³ This effect was largely due to a greater cognitive decline in the placebo group without an *APOE4* allele, with this effect being particularly prominent in the female patients.^{22,23} Indeed, in contrast to the results of the current analysis of metrifonate data, which showed no effects of gender, a significant three-way interaction among *APOE* genotype, gender, and treatment effect was observed for tacrine treatment.^{22,23} The factors contributing to divergent results of the analyses for these two different cholinesterase inhibitors are not known. However, they may include the following: the tacrine results occurring by chance; genotyping in the metrifonate studies versus *APOE* phenotyping in the tacrine study; analysis of a larger sample of metrifonate-treated than tacrine-treated patients; the different pharmacodynamic characteristics of metrifonate and tacrine; and the longer-lasting AChE inhibition achieved by metrifonate.

The current analysis of the influence of *APOE* genotype on AD progression suggests that although there may be a minor trend toward more rapid disease progression in patients who carry the *APOE4* alleles, the different *APOE* genotypes do not significantly influence the clinical course of the disease.

Two studies, one of which was a cholinesterase inhibitor clinical trial, suggested that AD patients with one or more *APOE4* alleles may actually progress less rapidly, a finding that contrasts with the results of the current study.^{23,38} However, most previously published studies have not shown any differences in the rates of disease progression as a function of *APOE* genotype. Kurz et al.¹⁹ prospectively evaluated 64 AD patients with respect to their cognitive performance over time and found that AD patients who were carriers of an *APOE4* allele declined cognitively at the same rate as those patients who were not carriers of an *APOE4* allele. Similarly, Growdon et al.²⁰ assessed 66 probable AD patients for up to 5.5 years with nine different cognitive tests and found that all patients deteriorated over time at a rate independent of the *APOE* genotype. A study of the relationship between *APOE4* allele copy number and the rate of cognitive decline in 86 probable AD patients was performed by Murphy et al.,²¹ who reported no strong association between the number of *APOE4* alleles and disease progression.

The inconsistencies in the reports of *APOE* genotype and relationships to rates of the progression of AD over time suggest that there may be no significant correlation. However, additional data from large patient populations would still be helpful to more definitively address this issue. In this regard, data from clinical trials and their extensions, reflecting specifically defined assessments repeated at regular intervals over years, may potentially be the most informative. Although it should be remembered that patients who enroll in clinical trials may not be exactly representative of the general population, these studies would generate the most scientifically valid data.

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Disclosure

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Ipsilesional intentional neglect and the effect of cueing

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Article abstract—Background: Contralesional hemispatial neglect may be induced by an attentional deficit where patients are inattentive to or unaware of stimuli in contralesional hemispace, an intentional deficit where patients are unable to act in or towards contralesional hemispace, or both. The deficits associated with ipsilesional neglect have not been as well characterized. Because cueing may be used as a rehabilitative assistive device, we wanted to learn whether the efficacy of an attentional or intentional cue was related to the type of bias. **Methods:** We studied a patient with a right frontotemporal stroke who had ipsilesional neglect by using a video apparatus that dissociates sensory-attentional and motor-intentional systems. We also performed a cueing experiment with primarily sensory-attentional cues (i.e., read the letter at the end of the line) and primarily motor-intentional cues (i.e., touch the end of the line). **Results and Conclusions:** Ipsilesional neglect was primarily a motor-intentional deficit with a motor-action bias to the left and a secondary sensory-attentional bias for stimuli to the right. With cueing we found a double dissociation: the rightwards motor-intentional cue improved the primary left-sided intentional bias and the leftwards sensory-attentional cue improved the secondary right-sided attentional bias. Effective rehabilitation strategies need to address both sensory-attentional and motor-intentional deficits in patients with neglect. **Key words:** Neglect—Attention—Intention—Cueing.

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Although most patients with unilateral or hemispatial neglect have a spatial bias toward the lesioned hemisphere (ipsilesional bias),¹ there have been several reports of patients who appear to show a contralesional bias on tasks such as line bisection.^{2–5} Patients with a bias toward contralesional hemispace demonstrate ipsilesional neglect. Patients with hemispatial neglect may be impaired because they have a sensory-attentional (ATT) bias, a motor-intentional (INT) bias, or both.^{6–10} The term *attentional bias* is used either if patients are unaware of stimuli on one side or if the stimuli on one side appear less salient than do the stimuli on the other side. Patients with neglect who have an intentional bias have a directional or hemispatial akinesia or hypokinesia and therefore fail to act or make hypometric movements in one direction. Whereas contralesional attentional neglect appears to be primarily associated with parietal lesions, contralesional intentional neglect appears to be primarily associated with frontal lesions.^{7–11}

To determine if a patient's primary bias on a given task is either attentional or intentional, it is necessary to alter the fundamental congruence of ATT input and INT output. We previously used an image-reversal paradigm contrasting ATT and INT bias in patients with spatial neglect¹¹ and normal subjects.¹² This image-reversal paradigm uses a video apparatus to contrast ATT systems directed toward one hemispace with INT systems directed toward the op-

posite hemispace. If the image is right-left reversed and the bias on the line-bisection task also is reversed, this would suggest ATT neglect. However, if the image is reversed and there is no reversal of the bias, the primary defect is INT neglect.

We studied a patient who had ipsilesional neglect from a right frontotemporal stroke to learn if this patient's ipsilesional deficits were caused by primarily an ATT or INT bias. Although a defect in one system would determine a primary bias, the other system may be responsible for a secondary bias. For example, a defect primarily resulting from an INT bias would present as a directional hypokinesia, but there might be a secondary, or less robust, component of inattention that also influences behavior. The secondary bias could be directed in the same or opposite direction as the primary bias.

Lateral cues on the line-bisection task may influence the severity of contralesional neglect.¹³ However, the effects of cueing have not been studied fully in ipsilateral neglect. Therefore, we also wanted to learn whether cueing would affect ipsilesional neglect. A lateral spatial cue might help the subject direct attention to that location. Alternatively, a cueing condition that involves limb movements may help activate and engage intentional systems necessary to prepare an organism for action. We posited that an attentional cue in the direction of an ATT deficit would improve an ATT bias and an intentional cue in the direction of an INT deficit would

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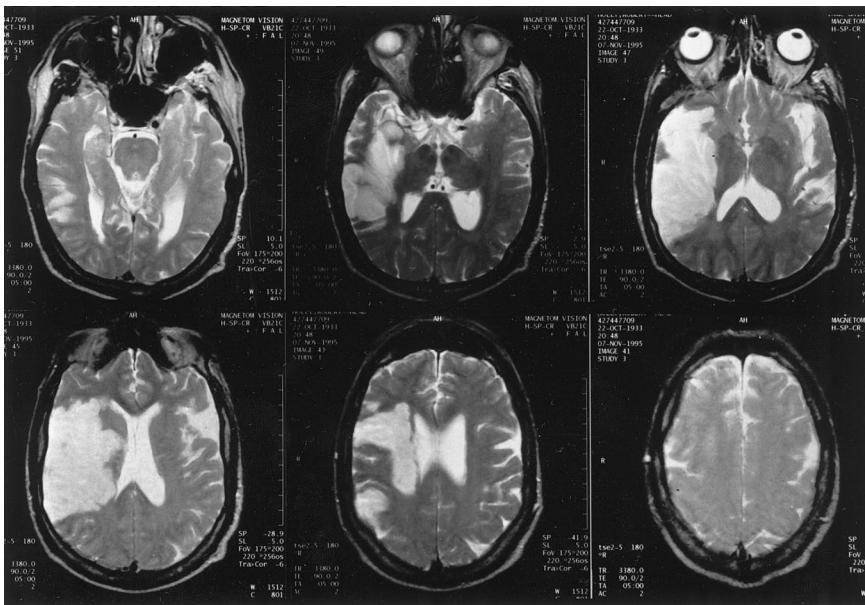


Figure 1. Brain MRI (T2-weighted) showing infarction in territory of the right middle cerebral artery involving dorsolateral frontal lobe and temporal lobe.

improve an INT bias. Because these two systems may be directed in either the same or opposite directions, the effect of attentional and intentional cueing conditions may be dissociable. Therefore, we administered a two-part cueing experiment with primarily ATT cues (i.e., read the letter at the end of the line) and primarily INT cues (i.e., touch the end of the line). Because neglect can be subtyped according to primary and secondary bias, ATT or INT cues may improve performance in the direction of these primary and secondary biases.

Case report. A 62-year-old, right-handed, retired shipyard worker with 12 years of education had a 15-year history of bipolar disorder well controlled with lithium. He had had a right-leg amputation below the knee for osteomyelitis. He presented with slurred speech and a left hemiparesis. His MRI was consistent with a right middle cerebral artery infarction involving predominantly temporal and dorsolateral frontal lobe regions (figure 1).

His neurologic examination revealed a left central facial palsy with a flaccid left hemiparesis. Otherwise, his cranial nerves were normal, including visual fields. Sensory examination revealed left hemibody hypesthesia (light touch and pinprick). His deep tendon reflexes were increased on the left with an extensor plantar response.

Cognitive examination revealed a strong left-sided bias on the line-bisection task. On target cancellation, he did not make errors but had more perseverative responses, in which he cancelled the same target more than once, on left-sided targets. He demonstrated frontal-lobe deficits including a manual and a visual grasp reflex.¹⁴ The visual grasp reflex was tested by asking the patient to "look at my nose and when you see my right or left hand move, you are to look at the opposite hand." The examiner placed his or her hands so that the examiner's left hand was in the patient's right visual field and vice-versa. The examiner then moved (flexed and extended) either the right or left hand. The order in which the hand was moved was randomized. An incorrect response was when the patient made saccadic movements toward the moving rather than

the stable hand. Our patient made errors on this task 50% of the time without a directional predominance. He performed well on "go/no go" testing ("When I raise one finger, you raise two, but when I raise two fingers, you raise no fingers") but had difficulty changing cognitive sets.¹⁵ On contrasting programs ("When I raise one finger, you raise two fingers. When I raise two fingers, you raise one finger"), he demonstrated echopraxia and perseverative behavior on 30% of trials. On the Controlled Word Association Test,¹⁶ a test of verbal fluency, he produced a total of only nine exemplars for the letters F, A, and S. He also was impaired on semantic category fluency (nine exemplars for animals, three exemplars for fruits). The patient was fully oriented except for the day of the week and the floor of the building. He recalled presidents back to President Carter. He registered three of three items and recalled three of three after distraction, and his serial threes ("subtract 3 from 100 and then continue to subtract by 3s") were intact. He was able to spell "world" forwards and backwards. Digit span ("repeat these numbers after me") was seven numbers forward. He had no motor impairment. His spontaneous speech was fluent with simplification of grammar, short mono-word answers, and mild dysarthria. There were no paraphasic errors. Naming, repetition, and comprehension were intact. Reading also was intact. He had no limb apraxia or right-left confusion. His finger naming was normal. He had perseverative behavior on a constructional task (interlocking pentagons).

Post-stroke psychiatric evaluation of the patient revealed depression, but there was no evidence of suicidal ideation. His thought process and content were intact with no delusions. He was treated with lithium and sertraline without side effects.

Methods. The following is a brief description of the video apparatus. More detailed descriptions of the testing methodology have been published elsewhere.^{11,12}

The testing procedure had two experimental conditions, direct and indirect. In the direct condition, the direction of sensory-attentional input was aligned with motor-intentional output (e.g., the left workspace was viewed on

Table Results of patient testing

Variable	Direct condition		Indirect condition	
	MLBE, mm (SD)	p Value	MLBE, mm (SD)	p Value
Neglect subtype (non-cued)*	-11.1 (16.7)	<0.0001	-39.6 (21.4)	<0.0001
Cueing				
Attention right†	8.7 (10.2)	<0.0001	-28.0 (25.4)	=0.154
Attention left†	-30.3 (20.4)	=0.004	-11.5 (34.0)	=0.005
Intention right†	-4.4 (10.7)	=0.187	-19.4 (32.3)	=0.045
Intention left†	-29.1 (13.8)	=0.002	-43.3 (20.1)	=0.639

* p Values reflect *t*-test comparison between direct and indirect condition.

† p Values reflect *t*-test comparison between each cued experimental condition (direct or indirect) and the same condition in the non-cued neglect subtype experiment (e.g., attention left/direct condition vs. noncued direct condition, attention left/indirect condition vs. noncued indirect condition).

the left side of a television monitor and the right workspace was viewed on the right side of a television monitor). In this direct condition, a videocamera was positioned above a clear, plexiglass surface (workspace) and connected to a television monitor resting in the midline directly beyond the workspace. A drape was placed on the front edge of the workspace, blocking the patient's direct view of the workspace (including both his hand and the test stimuli). His right hand was placed under the drape to perform a series of line-bisection tasks in the designated workspace while viewing his hand and the stimulus only on the monitor. The workspace was illuminated from above.

In the indirect condition, sensory-attentional input was uncoupled from motor-intentional output such that the right workspace was viewed on the left side of a television monitor and vice-versa. Furthermore, the direction of horizontal movement was uncoupled such that movement of the hand rightwards in the workspace was viewed as leftwards on the monitor and vice-versa. In the uncoupled or indirect condition, the videocamera was positioned below the clear, plexiglass surface, because the workspace was illuminated from above. The patient performed the identical bisection tasks in this condition, as he did in the direct condition. Backlighting allowed him to see his hand's shadow, which was transmitted to the television monitor. Vertical and radial dimensions remained unchanged in this condition.

The worksheet was centered in the workspace so that the line-bisection tasks were also centered on the monitor. The projection of the worksheet filled the entire screen to eliminate inadvertent cues. The patient's head and body were maintained in a midline position such that the midsagittal plane of the patient was aligned with the midsagittal plane of the monitor, workspace, worksheet, and stimuli. He performed all tasks with his right hand. His eye movements were not restricted, and he had no time limit.

The patient was tested within the first week post-stroke, with each session lasting no longer than 1 hour. On all line-bisection tasks, the mean line-bisection error (MLBE) was measured to the nearest millimeter. By convention, leftwards errors were scored as negative and rightward errors as positive. All results are reported according to the actual bisection error on the worksheet, as

opposed to the monitor. Statistical calculations were done with *t*-tests comparing the MLBE in the direct condition to the MLBE in the indirect condition. In the cueing experiment, we were interested in the effect of attentional and intentional cueing with respect to the noncued performance. We therefore used *t*-tests to compare the MLBE in each condition (direct and indirect) to the same condition in the noncued neglect experiment.

Attentional versus intentional neglect experiment. The patient was shown a series of 20 lines in both the direct and indirect conditions. Each line (230 × 3 mm) was positioned horizontally in the center of a page (215 × 280 mm). He was instructed to place a single bisection mark on the exact center of each line.

As shown in the table, in the direct condition, the patient demonstrated right hemispatial (ipsilesional) neglect on the line-bisection task. In the indirect condition, his error was significantly further leftwards. These results suggest that he had primarily motor-intentional ipsilesional neglect, with a bias to act in and toward left space or an inability to act in or towards right hemisphere. He was able to perceive right hemisphere, because his action in left hemiworkspace was viewed as right sensory-attentional space on the monitor.

The patient's secondary ATT bias was focused in the opposite direction (rightward) from the primarily leftward INT bias. In the indirect condition, if ATT also was biased leftwards, then the patient's motor action would have been drawn rightwards, thus decreasing his MLBE compared with the direct condition (figure 2). In contrast, if ATT was biased rightwards, then his motor action would have been driven further leftwards, thus increasing his error compared to the direct condition (see figure 2). If there was no secondary bias, then the patient's performance would not have changed from the direct to the indirect condition. Because his error increased significantly from the direct to the indirect condition, we conclude that his primary INT bias was directed leftward and his secondary ATT bias was in the opposite direction (rightward).

Cueing experiment. Attentional cues. The patient was shown a series of 30 lines in both the direct and indirect conditions. Each line (230 × 3 mm) was positioned horizontally in the center of a page (215 × 280 mm). Each line had a letter (R or K) positioned at each end such that the worksheet was perceptually symmetric. He was in-

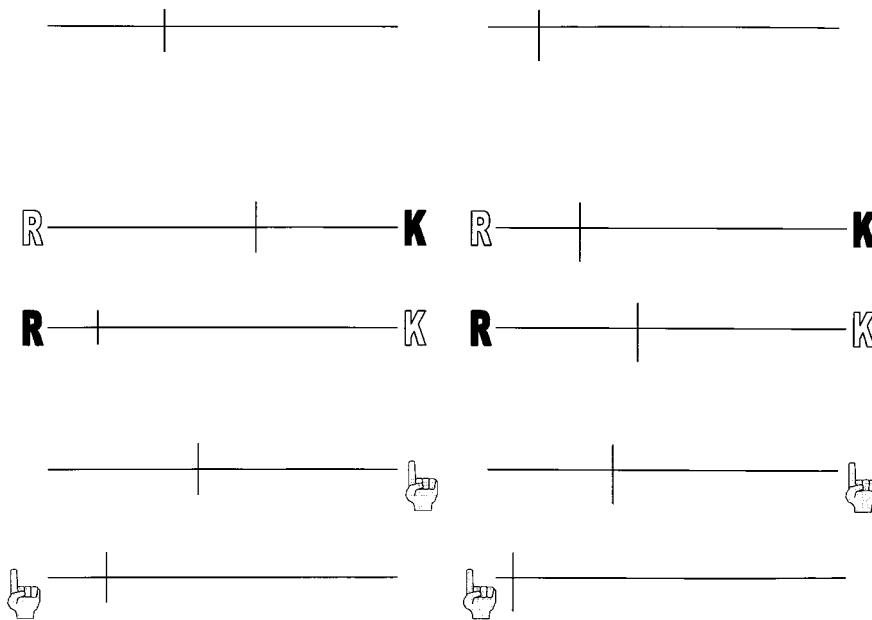
DIRECT**INDIRECT**

Figure 2. Example of patient's performance. Bisects represent mean line-bisection error in each condition. In the attentional experiment, letter stimuli were identical on each side of the line, shading in figure for emphasis only. Figure is not to scale. From top to bottom: noncued neglect subtype experiment, attentional cue right, attentional cue left, intentional cue right, intentional cue left.

structed to first read the letter at the right or the left end of the line and then place a single bisection mark on the exact center of each line. The order of right and left cues was pseudorandomized across trials.

In the direct condition, when the patient read the letter on the left side of the line his error was drawn significantly leftwards and when he read the letter on the right side of the line he was drawn significantly rightwards. In the indirect condition, when he read the letter displayed on the left side of the monitor that was on the right in actual workspace, his MLBE significantly improved (see figure 2). In contrast, when he read the letter on the right side of the monitor hemispace, his MLBE was unchanged.

Intentional cues. The patient was shown a series of 30 lines in the direct condition and 22 lines in the indirect condition. Each line (230×3 mm) was positioned horizontally in the center of a page (215×280 mm). He was instructed to first mark the right or the left end of the line and then place a single bisection mark on the exact center of each line. The order of right and left cues was pseudorandomized across trials.

As shown in the table, in the direct condition, when the patient first touched the left end of the line his MLBE shifted significantly leftwards. When he touched the right end of the line, his MLBE did not change. In the indirect condition, when he first touched the left end of the line, in actual workspace his MLBE did not change. When he first touched the right end of the line in actual workspace, his MLBE significantly improved.

Discussion. We studied a patient with ipsilateral neglect from a right frontal-subcortical lesion. Previous reports have suggested that ipsilateral neglect may result from disinhibition of contralateral motor-intentional approach behaviors similar to the visual grasp and utilization response seen in patients with frontal-lobe injury.^{3,14,17} However, this motor-intentional hypothesis has not been tested

directly. Our study provides a direct evaluation of the ATT and INT biases associated with ipsilesional neglect.

Our patient demonstrated primarily INT neglect of right hemispace or a motor-action bias toward left hemispace. The indirect condition of our paradigm created an incongruous set wherein the INT system and the ATT system were dissociated such that the direction of movement in motor-intentional workspace was opposite the direction of movement in sensory-attentional space. In the indirect condition, when he made leftward errors in the workspace he was actually viewing his performance in right hemispace on the television monitor. Thus, his primary spatial bias reflected a bias to act in or toward left hemispace or an inability to act in or toward right hemispace despite adequate awareness of right hemispace.

We posited that our patient also would have a secondary ATT bias that might be focused in the same direction (toward left hemispace/ipsilesional neglect) or the opposite direction (toward right hemispace/contralateral neglect) as the primary INT bias. In the indirect condition, if ATT was biased toward left hemispace, then his MLBE would have been driven rightwards and his performance on the line bisection task would have improved. In the indirect condition his MLBE deviated further leftwards, suggesting that his ATT bias was directed towards right hemispace. Thus, our patient demonstrated primary ipsilesional INT neglect and secondary contralateral ATT neglect.

A previous report described a patient with a right frontal lobe lesion who initially demonstrated contralateral visual inattention and ocular directional akinesia on a visual crossed-response test.¹⁴ Over

time, that patient's visual inattention resolved but his directional ocular-motor neglect remained. Subsequently, the contralateral motor neglect also resolved and gave way to contralesional ocular-motor approach (visual grasp). This differential resolution of ATT and INT biases in the oculo-motor system is similar to other patients who have shown ipsilesional neglect on a line-bisection task.³ We would predict that a primary INT bias would remain more robust as a secondary ATT bias resolved over time. Unfortunately, we do not have direct evidence of this differential resolution of ATT or INT bias in our patient.

The intentional system provides information to the motor system regarding goal-directed actions. A widely distributed frontal–subcortical network is important for mediating intentional motor behaviors, and the right hemisphere may be dominant for directing those behaviors toward both sides of hemispace.^{18,19} Contralesional motor neglect from dorsolateral frontal lesions has been demonstrated in monkeys.²⁰ Dorsolateral frontal-lobe lesions in humans have been associated with motor impersistence,²¹ defective oculo-motor response inhibition,¹⁴ and intentional neglect.²² Although the pathophysiology of our patient's ipsilesional intentional and contralateral attentional neglect remains to be fully determined, we suspect that these behaviors, at least in part, are related to the frontal lobe's influence on other systems. The attentional and intentional systems are not anatomically or functionally separated from each other. For example, the frontal lobes, which play a critical role in mediating intention, have strong reciprocal anatomic connections with the parietal lobes, which play a critical role in spatially directed attention. Perhaps acute frontal lesions, in addition to producing a contralesional intention deficit, also impair parietal-lobe function, thereby also inducing contralateral inattention. The frontal lobes also inhibit subcortical systems such as the superior colliculus. After these subcortical intentional systems, such as the superior colliculus, recover from the diaschisis associated with cortical injury, they may mediate contralateral ocular-motor approach behaviors.²³ Perhaps the release of the uninjured basal ganglia or the ipsilateral parietal lobe from frontal-lobe control may be important in these motor-approach behaviors, and the combination of contralateral approach behaviors and inattention may account for the findings in our patient.

Alternatively, because the right hemisphere may be dominant for attention to both right and left hemispace,^{24–26} impairment of right-hemisphere attentional systems may allow for an attentional bias towards right hemispace mediated by left-hemisphere attentional systems that are directed predominantly rightwards. Thus, the opposing directional biases in our patient may have been partially mediated by opposing hemispheres.

Because our patient had ipsilateral intentional neglect and contralesional attentional neglect, we

wanted to learn how primarily ATT and INT cueing would affect our patient's spatial bias. When directed first to read a letter at one end of a line and then to bisect the line, both normal subjects²⁷ and patients with neglect¹³ deviate toward the side of the letter compared with control noncue conditions. The act of directing attention to one hemispace biases performance toward that hemispace.²⁸ However, reading the letter on one side of the line also requires eye movements, and the eye movements associated with perceiving the target letter may engage intentional mechanisms that prepare the subject for action toward that part of space. Pointing to the end of a line also requires coordination of ATT and INT systems. In our letter-reading and pointing (cueing) experiments, despite the likely coactivation of both ATT and INT systems, we presumed that reading a letter would more strongly activate ATT systems and that marking the end of the line would more strongly activate INT systems. If these presumptions were correct, then attentional cues (read the letter) towards left hemispace would improve his contralesional ATT neglect and intentional cues (mark the end of the line) toward right hemispace would improve his ipsilesional INT neglect. The results of line bisections in the indirect condition of the cueing experiments support this dissociation.

Patients with ipsilesional neglect following unilateral right-hemisphere lesions may recover from neglect using compensatory visual scanning strategies. Because the right hemisphere is dominant for attention, one hypothesis has been that unilateral right-sided lesions may compromise these compensatory strategies because of attentional deficits of both right and left hemispace.⁵ In the direct condition of our cueing experiments, our patient was influenced by attentional cues to both sides of space, but he was only influenced by a left-sided intentional cue. This differentiation argues more strongly for a primary intentional deficit of right hemisphere or an intentional bias toward left hemispace. The indirect condition of our cueing experiments further dissociates directional attentional neglect of left hemispace as a secondary influence on a primary intentional neglect of right hemispace. These lateralized ATT and INT deficits do not directly address the issue of compensatory rehabilitation strategies in this patient. In our view, visual scanning strategies may activate both attentional (visual) and intentional (eye-movement) systems, and it is the interaction of directional biases that determine an individual's improvement with rehabilitation.

As hypothesized in several recent investigations, anterior lesions (frontal–subcortical) may predominantly produce INT neglect, and posterior lesions (temporoparietal) may produce ATT neglect.^{7,11,29–31} Because the two systems are interconnected,³² damage to one system may produce a primary spatial bias with the second system providing a secondary influence. Our study dissociates attentional and intentional biases in a patient with right-sided ipsile-

sional neglect from a frontotemporal stroke. The double dissociation observed in the cueing task strengthens the hypothesis that this patient had a primary INT neglect and secondary ATT neglect directed towards opposing hemisphere.

Cueing may be used as a rehabilitative or assistive technique. Determining whether neglect is contralateral or ipsilesional and is attentional or intentional may be critical in planning effective rehabilitation strategies.

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Left unilateral neglect or right hyperattention?

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Article abstract—Background: Contradictory interpretations of left unilateral neglect suggest that it reflects either decreased attention toward the left or increased attention toward the right. According to the right-hyperattention postulate, increasing severity of neglect should result from an increasingly stronger bias toward the right. Thus, response times to right-sided targets should become progressively faster as neglect increases in severity across patients. The left-hypoattention postulate predicts that as neglect increases, progressively less-attentional resources are deployed in both hemispheres. Thus, response times to right targets should progressively increase with increasing neglect. **Methods:** We analyzed the distribution of manual response times to left- and right-sided targets in 24 patients with right hemisphere lesions and varying degrees of left neglect. **Results:** Not only the responses to left targets but also those to right targets became progressively slower as neglect increased, consistent with the hypoattention account. However, the two regression lines were not parallel. With increasing neglect, responses to left targets increased more steeply than those to right targets did. **Conclusions:** A rightward attentional bias is present in patients with left neglect, together with left hypoattention. However, this rightward bias is one of defective, and not enhanced, attention. **Key words:** Right brain damaged—Reaction time.

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Patients with left unilateral neglect fail to notice events occurring on their left. They bump into furniture on their left side, do not eat from the left part of the dish, and do not answer to people standing on their left. The first impression that one gathers from the observation of these patients is that they pay no attention to half of their visual world. However, the simple statement that unilateral brain damage may determine a deficit of attention for the contralateral hemisphere does not capture one of the most striking aspects of neglect, namely, that neglect is more common, severe, and persistent after right hemisphere than after left hemisphere damage. To account for this basic characteristic of neglect, Heilman and Van Den Abell¹ proposed that the left hemisphere attends to contralateral space whereas the right attends to both contralateral and ipsilateral hemispheres. Thus, left hemispheric damage could be compensated for by right hemispheric attentional mechanisms, thereby only rarely provoking right neglect. Conversely, right cerebral damage would cause left neglect because the left hemisphere is unable to attend to the left hemisphere. Thus, right hemisphere lesions should determine a severe deficit in attention for the contralateral hemisphere but also a milder ipsilateral deficit, because less-attentional resources are now deployed in the right hemisphere.² Consistent with this notion, left neglect patients may be impaired also in the right hemisphere.^{2–5}

In a different account of neglect, Kinsbourne^{6,7} posited that each hemisphere shifts attention toward

the contralateral hemisphere by inhibiting the other hemisphere (the opponent processor model). In the normal brain, there is a tendency to rightward orienting supported by the left hemisphere. Right hemisphere lesions determine left neglect by exaggerating this physiologic rightward bias. Left hemisphere lesions would only rarely provoke right neglect because they release a right hemisphere attentional vector, which is less powerful than the left one. Left neglect does not reflect an attentional deficit but an attentional bias consisting of increased attention to the right. This bias is coupled with an abnormally tight focus of attention, which deprives patients of the possibility of a more general overview of the visual scene.⁷ It follows that the sagittal midline plays no crucial role in neglect, every stimulus location being likely to be neglected if it is “left of” some other stimulus. This would account for the occurring of right omissions in left neglect,^{2–5} because patients’ attention would be captured by stimuli lying further to the right of those omitted.

These models have inspired major research efforts.^{7,8} Here we focus on competing predictions stemming from these models on the distribution of manual reaction times (RTs) to lateralized visual stimuli. When responding to horizontally aligned stimuli, patients with left neglect should be slower for left-sided than for right-sided stimuli according to both hypotheses. However, according to the right-hyperattention model, this asymmetry should be observed, even when all the stimuli are presented in

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the right visual hemifield. Consistent with this prediction, Ládavas et al.⁹ found that when presented with stimuli horizontally aligned in the right hemifield, patients with left neglect responded faster to right-sided than to left-sided targets. Right brain-damaged (RBD) patients without neglect, on the contrary, were faster for left-sided than for right-sided stimuli, probably because left targets appeared closer to the fovea. Of particular interest was the finding that neglect patients were faster for right targets than RBD patients without neglect. Neglect patients' attention for right targets seemed thus enhanced with respect to RBD control subjects, consistent with the right-hyperattention model. As Ládavas et al.⁹ pointed out, according to this model, neglect patients should be faster for right-sided stimuli, even with respect to normal individuals without brain damage; this, however, would be an unlikely result, given that right brain damage lesions cause a deficit in arousal.¹⁰ Indeed, subsequent RT studies¹¹⁻¹⁴ invariably found that left neglect patients were slower than control subjects when responding to right (ipsilesional) stimuli.

Another possibility consistent with these findings is that an important component of left neglect is a rightward bias of *defective* attention.^{11,12,15} This could explain the evidence of an attentional gradient favoring events situated to the relative right of other events, coupled with the finding that even the rightmost events cannot elicit normal attentional processing.

Because neglect is not an all-or-none phenomenon but may vary in severity across patients, further testing of these issues is allowed. The left-hypoattention and the right-hyperattention models make opposite predictions concerning the distribution of RTs to right targets with increasing degrees of neglect. According to the hypoattention model, which postulates a bilateral attentional representation in the right hemisphere, severe damage to this system should also produce relative inattention for the right side, now attended to solely by the left hemisphere.² Thus, the hypoattention model predicts that responses to right-sided stimuli should be progressively slower as left neglect increases, paralleling the slowing of responses to targets presented in the left hemispace.

Conversely, the hyperattention model posits that left neglect is a direct consequence of enhanced attention to the right; thus, increasing severity of neglect should reflect an increasing amount of rightward bias, leading, in turn, to progressively faster RTs to right-sided stimuli.

If a rightward bias of impaired attention is present in neglect, then the relative weight of rightward attentional attraction and of attentional impairment should determine the direction of the RT distribution for right targets. RTs to right targets should progressively decrease with increasing neglect if rightward attentional attraction prevails or progressively increase if attentional impairment dominates. In this latter case, the variation of the RTs to right targets as a function of neglect severity should be less steep than the corresponding varia-

tion of RTs to left targets, because both rightward attentional attraction (leading to faster RTs) and attentional impairment (causing slower RTs) would increase with increasing neglect.

If, finally, a nondirectional attentional deficit, such as defective arousal, is aspecifically associated with right brain damage and not with neglect, its amount (and, therefore, its influence on RTs) should not correlate with the severity of neglect; no lawful relationship should therefore emerge between these two variables.

To test these predictions, we examined a group of RBD patients with left neglect using a quantitative measure of the amount of their spatial bias in paper-and-pencil tests and a task of speeded manual responses to lateralized visual stimuli. Hence, we were able to explore the distribution of RTs to left and right targets as a function of the severity of left neglect.

Methods. *Patients.* Twenty-four patients with left unilateral neglect consented to participate in the study. All had CT or MRI evidence of unilateral lesions in the right hemisphere (see table).

Tests of unilateral neglect. The presence and severity of unilateral neglect were assessed by using a battery of visuospatial tests,¹⁶ which included tasks of line cancellation,¹⁷ identification of overlapping figures,¹⁵ and line bisection.¹⁸ To measure subjects' spatial bias independent of their overall performance level, laterality scores derived from Bryden and Sprott¹⁹ were used. The procedure was described elsewhere.¹⁶ To summarize, the direction and amount of spatial bias were estimated by the following formula:

$$\lambda = \ln(X_R/X_L)$$

Values of X_R were computed by adding the following:

1. The number of items identified on the right side of the overlapping figures test (max = 10).
2. The number of lines canceled on the right half of the page of the line cancellation test (max = 30). Some patients with severe neglect (see table, Patients 18-24) started the test from the right side and did not cross the midline of the sheet; for these patients, the value (2) was replaced by the total number of neglected lines (max = 59).
3. The sum of the number of segments to the left of each subject's bisectors (max = 42) in the line bisection task, in which the lines were divided into 20-mm segments.

Values of X_L were computed in analogous fashion (i.e., by adding the items of the left-sided identified superimposed figure to the number of left-sided cancelled lines and to the number of segments to the right of line bisection). Patients were considered to be affected by left neglect and included in the current study when their λ score exceeded the cutoff score defined by the mean +3 SDs of 30 control subjects' performance¹⁶ (i.e., +0.104).

RT test. After a previously described procedure,¹³ patients sat in front of a computer monitor at a distance of approximately 50 cm. Three horizontally arranged black circles, 14 mm in diameter, were displayed, the central circle being located at the center of the screen. Distance between

Table Demographic and clinical characteristics of patients, who are ordered according to increasing severity of left neglect

Patient no.	Sex, age, years of schooling	Onset of illness, d	Etiology	Locus of lesion	Visual field
1	M, 61, 8	135	Traumatic	TP	Normal
2	F, 66, 11	20	Neoplastic	TP, Th	Left extinction
3	F, 82, 7	7	Hemorrhagic	P	Left extinction
4	M, 77, 12	30	Ischemic	FP	Left extinction
5	M, 67, 18	37	Ischemic	Th	LSQ
6	M, 43, 8	119	Hemorrhagic	IC, Th	Normal
7	M, 67, 8	141	Hemorrhagic	FPT	Left extinction
8	M, 76, 5	15	Ischemic	P	LIQ + left extinction
9	F, 53, 7	76	Ischemic	FP	Left extinction
10	M, 46, 6	111	Ischemic	TFP	Left extinction
11	M, 80, 17	173	Ischemic	TO	LIQ
12	M, 69, 13	251	Ischemic	IC, BG	LH
13	M, 65, 12	52	Hemorrhagic	FP	Left extinction
14	M, 76, 7	4	Ischemic	TO	LH
15	M, 71, 7	20	Ischemic	FP	LIQ + left extinction
16	M, 62, 12	449	Hemorrhagic	TO	LH
17	F, 62, 15	113	Ischemic	O, Th	LH
18	M, 66, 13	12	Ischemic	FP	Left extinction
19	M, 73, 9	48	Hemorrhagic	FP	Normal
20	M, 63, 9	91	Hemorrhagic	FT	Left extinction
21	M, 43, 11	44	Traumatic	TP	LH
22	F, 69, 4	133	Ischemic	IC	LH
23	F, 73, 8	244	Ischemic	FP	LH
24	M, 53, 12	75	Ischemic	BG, IC	MA

F = frontal; T = temporal; P = parietal; O = occipital; Th = thalamic; IC = internal capsule; BG = basal ganglia; LIQ = left inferior quadrantanopia; LSQ = left superior quadrantanopia; LH = left hemianopia (with macular sparing); MA = rightward magnetic attraction of gaze on confrontation testing.

circles was 23 mm. During the test, the circles were always present on the screen. After an interval of 2000 msec, one of the circles became gray (target). When a right- or a left-sided target appeared, patients had to respond by pressing the computer spacebar with the index finger of the right hand as quickly as possible. Patients had to refrain from responding when the middle circle became gray (catch trials). Response time was measured from target onset to key press. The target disappeared when a response was made or after 5000 msec. After 1 block of 6 practice trials, 10 test blocks were presented, each including 4 right-sided and 4 left-sided trials plus 1 catch trial. The order of trials within a block was randomized. RTs ranging from 150 to 4500 msec were retained for subsequent analysis. RTs exceeding this range were considered as omissions.

Results. *Reaction times.* Figure 1 displays each patient's mean response time to left- and right-sided targets as a function of the severity of left neglect. RTs to left targets increased monotonically with increasing severity of left neglect, $F(1, 22) = 21.53, p < 0.001$, with a slope of 606 msec/U of λ score. More important, also RTs to right targets increased with severity of left neglect, $F(1, 22) = 8.28, p < 0.01$, with a slope of 201 msec/U. Thus, as can be seen

in figure 1, the two regression lines are not parallel; as neglect increases, RTs to left targets increase more steeply than do RTs to right targets, as confirmed by the difference found between the two slopes, $t(44) = 2.73, p < 0.01$. In addition, the intercept of the regression line for RTs to left targets (1187 msec) was larger than the intercept of the line for right targets (887 msec), $t(44) = 2.46, p < 0.02$. This result predicts that our RT test would disclose a spatial bias (advantage for right versus left targets), even in the absence of biased performance on paper-and-pencil tests (λ score = 0); this was confirmed in other studies with the same RT paradigm,^{12,13} including RBD patients without clinical signs of neglect.

Fifteen age-matched control subjects¹³ had response latencies of 556 msec for left targets and 566 msec for right targets on the same task. Responses to right targets were 489 msec faster for control subjects than for neglect patients of the current study, $t(37) = -6.23, p < 0.0001$. When only the 11 patients with less-severe neglect ($\lambda < 0.5$) were taken into consideration, they were still 398 msec slower than control subjects for right targets, $t(24) = -6.18, p < 0.0001$.

These findings provide evidence against the presence of right hyperattention in our series of neglect patients. How-

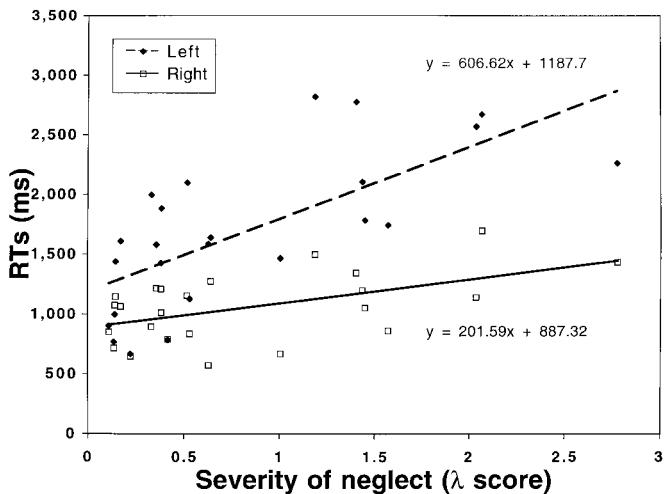


Figure 1. Regression plot of reaction times to left (filled symbols) and right (open symbols) targets as a function of the severity of left neglect (λ score).

ever, the possibility remains that right hyperattention existed only in a subgroup of patients, whose fast responses to right targets might have diluted the effect of hypoattention on RTs, thus producing a regression line that had the expected positive slope but to a lesser degree. To address this possibility, we selected the 10 patients with the fastest RTs to right targets and compared these RTs with those produced by age-matched control groups with right hemisphere lesions but no neglect¹⁸ ($n = 12$) and without neurologic impairment¹³ ($n = 15$). Even for this subgroup of neglect patients, RTs to right targets (mean, 784 msec; SD, 132 msec) were slower than those of control subjects [$t(23) = -4.05$, $p < 0.001$] and not faster than those of RBD patients without neglect (mean, 811 msec; SD, 263 msec; $t < 1$). These results argue against the presence in our series of a subgroup of neglect patients with right hyperattention.

Accuracy. By leaving the target on the screen for a long time (5 seconds) before response, we aimed at maximizing the accuracy of response in this simple target detection test. However, omissions did occur. Figure 2 displays the percentage of omissions as a function of the severity of neglect.

More left targets were omitted as neglect became more severe, $F(1, 22) = 21.53$, $p < 0.001$; the omission rate increased by 16% per unit of λ score. A tendency in the same direction was also present for right targets, whose omissions increased by 0.3% per unit of λ score, but this effect did not reach significance ($F < 1$).

Discussion. Taking advantage of quantitative evaluations of spatial bias, we were able to test alternative accounts of left unilateral neglect. We found that RTs to lateralized targets presented a lawful relationship with the severity of neglect as estimated by a laterality score. With increasing degrees of left neglect, not only RTs to left targets, but also RTs to right targets tended to increase. Results for accuracy of response were in the same direction, thus showing that patients were not trading speed for accuracy. Moreover, neglect patients (and even those with less-severe neglect) were definitely slower than control subjects when responding to both left and right tar-

gets. This pattern of results is more consistent with the left-hypoattention model¹ than with the right-hyperattention model^{6,7} and suggests that the rightward attentional bias is a consequence, and not the cause, of the contralateral attentional deficit; attention would be biased toward the right only because the left-directing processor is hypoactive.⁸

However, if left hypoattention simply "spills over" to the right side as neglect becomes more severe, we would expect the RT regression line for left targets and that for right targets to be parallel. This was not the case, because as neglect increases, RTs to left targets increase more steeply than do RTs to right targets (figure 1). Thus, our results are best accounted for by a mixed model of neglect in which both a rightward attentional bias and defective attention play a role, but the attentional deficit prevails over the rightward bias. Previous studies suggested that unilateral neglect results from an association of several attentional impairments.^{12,15,20,21} Our results are consistent with this notion and suggest that rightward attentional bias and defective attention concur in determining neglect severity.

One might argue that patients with severe neglect in our series did have increased attention to the right but were paying attention to spatial locations situated to the right of the entire stimulus array. If so, increasing severity of neglect would produce increasing RTs to right targets, because these targets would lay more to the left of the attentional focus. This account is unlikely because there was no object right of the right-most stimulus that could attract patients' attention; informal observation of eye movements confirmed that during the course of the test, patients looked at the stimulus array and never to the right of it. Furthermore, by such an account, there should have been degrees of neglect corresponding to an exact location of the attentional focus on the right target, thus leading to fast RTs to right targets. If so, when contrasted with neglect severity, RTs to right targets would have as-

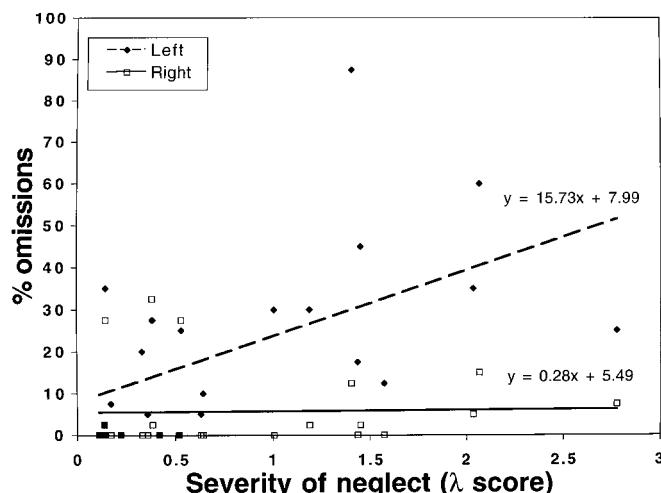


Figure 2. Regression plot of percentages of omissions to left (filled symbols) and right (open symbols) targets as a function of the severity of left neglect (λ score).

sumed a U-shaped curve and not the regression line apparent in figure 1.

Note that our measure of severity of neglect in paper-and-pencil tests was a laterality score that was independent of the overall level of performance. Thus, for example, increasing right-sided omissions on the cancellation test or the overlapping figure task with equal numbers of left omissions would decrease the amount of the score. Consequently, a nonlateralized pattern of omissions in paper-and-pencil tests, such as the one that would result from a nonspecific deficit in arousal or vigilance, could not inflate the neglect score. It follows from these considerations that the positive correlation that we found between the neglect score and the amount of RTs to both left and right targets cannot simply result from an arousal deficit aspecifically associated with right brain damage.¹⁰

The opponent processor model by Kinsbourne^{6,7} has been important in stressing crucial aspects of left neglect, such as the "magnetic attraction" of attention exerted by right-sided objects, a prediction since confirmed in several laboratories.^{11,15,22,23} What the current results suggest is that this spatial bias is not one of increased or normal attention but one of defective attention. This notion is consistent with PET data showing a widespread hypometabolism in both the lesioned and the intact hemisphere in neglect²⁴ and perhaps best exemplified by the performance of some patients with severe left neglect on line cancellation tasks. These patients not only neglect lines on the left side but also perseverate in that they cross out several times the lines situated in the rightmost extremity of the sheet. Thus, patients' attention is repeatedly attracted by the rightmost lines; however, their attention is insufficient to recognize that these lines are already crossed.

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Cerebral lateralization

Relationship of language and ideomotor praxis

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Article abstract—*Objective:* To determine the relationship of language lateralization and hand preference to praxis performance following left and right hemispheric amobarbital-induced inactivations. *Background:* Patients who are aphasic from left cerebral dysfunction also frequently exhibit ideomotor apraxia in which they make temporal, spatial, and postural errors of learned skilled movements. However, hemispheric lateralization of the systems mediating ideomotor praxis in patients with atypical cerebral language dominance (i.e., bilateral or right hemispheric language function) remains uncertain. *Methods:* Subjects included 90 patients with intractable seizures who were undergoing the intracarotid amobarbital procedure (IAP) as part of their preoperative evaluation for epilepsy surgery. Hand preference was determined by the Benton Handedness Questionnaire. Praxis was assessed by the subject's performance when pantomiming the use of four pictured tools. *Results:* During left IAP, patients with typical language dominance made more ideomotor apraxic errors than did patients with atypical language dominance. During right IAP, patients with atypical language dominance made more ideomotor apraxic errors than did patients with typical language dominance. Overall, patients with atypical language dominance made fewer ideomotor apraxic errors than did patients with typical language dominance. These relationships were present irrespective of hand preference. *Conclusions:* Language dominance is more closely associated with the laterality of temporal and spatial movement representations (i.e., ideomotor praxis dominance) than is hand preference. Patients with atypical language dominance exhibit more bilateral cerebral distribution of both language and praxis function. **Key words:** Apraxia—Aphasia—Lateralization—Wada test.

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Praxis is the ability to perform learned skilled movements.¹ In 1865, Broca commented that “a great many mechanical actions are controlled mainly or even exclusively by the left hemisphere of the brain. . . Just as we control movements in writing, drawing, embroidering, etc, with the left hemisphere, so we speak with the left hemisphere. It is a habit that we acquire in our early childhood.”² Liepmann^{3,4} first described the behavioral mechanisms of apraxia and showed that the highest incidence of what he termed ideomotor apraxia occurred in patients with left cerebral stroke and aphasia. Praxis and language functions can be dissociated but often occur concurrently because the cortical regions mediating these functions overlap.^{5,6} As a group, patients with atypical language dominance exhibit more bihemispheric distribution of language function.⁷ However, the left/right cerebral hemispheric mediation of praxis functions in patients with atypical language representation is uncertain.

Ideomotor apraxia is a disorder of production of learned skilled movements that cannot be explained by weakness, deafferentation, abnormalities of posture and tone, involuntary movement disorder, or an inability to comprehend.¹ Dexterity, including independent finger movements and fine motor control, may be normal, and the greatest difficulty that these

patients with apraxia have is performing transitive movements (i.e., showing use of a tool or instrument). Deficits may improve with imitation or with use of the real tool or instrument. A variety of production errors may be seen with ideomotor apraxia, including substitution of body part as the tool, perseverations, sequencing errors, timing errors (e.g., delay onset or incorrect pause or speed), and spatial errors. The types of spatial errors include postural (e.g., position hand incorrectly), spatial orientation (e.g., incorrect position of the limb), and spatial movement (e.g., activate wrong joint). Liepmann⁴ also described two other forms of apraxia: limb-kinetic apraxia, in which there is a loss of deftness or fine motor control, and ideational apraxia, in which patients cannot correctly sequence a series of acts leading to a goal (e.g., making a sandwich).

As part of the preoperative evaluation for epilepsy surgery, patients routinely undergo the intracarotid amobarbital procedure (IAP or Wada test).⁸ During the IAP, an intracarotid barbiturate injection transiently anesthetizes most of the ipsilateral cerebral hemisphere. Thus, the IAP provides the opportunity to examine the contribution of each hemisphere to both language and praxis function in each individual. In a prior study, when the ultra-short-acting barbiturate methohexitol was used for the Wada test

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in nine dextrals with left language dominance, ideomotor apraxia was associated with left hemispheric anesthesia in seven patients but was observed bilaterally in two patients.⁹ In the current study, we sought to extend these findings by examining the relationship between language dominance, hand preference, and the laterality of movement representations, as determined by the presence of ideomotor apraxia.

Methods. *Subjects.* There were 90 subjects with intractable seizures who were undergoing the IAP as part of their routine preoperative evaluation for epilepsy surgery. The study was conducted under Institutional Review Board approval. Each patient gave informed consent for the IAP and all related procedures. Hand preference was determined by the Benton Handedness Questionnaire.¹⁰ Patients with a score of 8 or greater were classified as dextral. Language dominance was characterized as typical (i.e., left cerebral) or atypical (i.e., right or bilateral cerebral) on the basis of the IAP as we described previously.⁸ Demographic data were as follows: mean age = 32 years (SD = 10 years); 39 women, 51 men; 79 dextrals, 11 nondextrals.

Procedure. Each intracarotid artery was cannulated via a transfemoral approach. After arteriography, 100 mg amobarbital was injected by hand over 4 seconds while the subject was counting from 1 to 20 with his or her hands raised in the air. The presence of speech arrest, motor strength/tone, and simple language comprehension was assessed initially followed by presentation of memory objects and then a more extensive evaluation of language function. Motor strength was then reassessed before the praxis test. Further details of the IAP assessment are available elsewhere.⁸

To assess praxis, patients were sequentially presented with four line drawings (i.e., key, screwdriver, scissors, and saw). With each drawing, patients were asked to show with their right and left hands how they would use each object. Order of hand testing (i.e., left/right and ipsilateral/contralateral) was balanced across tasks. Before the IAP, subjects performed the tasks to familiarize themselves with the tasks and to ensure that there was no baseline apraxia. Praxis was assessed by a behavioral neurologist and neuropsychologist based on performance of the four praxis tasks using a 0-to-3-point scale: 3 = normal, 2 = minor errors (e.g., slightly clumsy), 1 = moderate errors (e.g., body part substitution, spatial or timing errors), 0 = severe errors (e.g., perseverations, wrong item, unrecognizable movement, no response). There was a >95% agreement on the ratings, and differences were resolved by review of the IAP videotape. Performance scores were summed across the four trials for each hand in each condition (i.e., left and right IAP). The maximum best possible score for each hand in each condition was 12.

Analyses. The patients with typical (i.e., left cerebral) and atypical (i.e., right or bilateral) language dominance were compared using a 2- (language group: typical versus atypical) \times 2- (hand: left versus right) \times 2 (IAP injection: left versus right) analysis of variance. Indicated follow-up analyses were conducted along with a separate 2- (hand: L/R) \times 2- (IAP injection: L/R) analysis of variance for the typical language dominance group alone. In addition, the

type of performance errors was determined for the typical and atypical language dominance groups. The apraxic errors were characterized as limb-kinetic (e.g., slight clumsiness or loss of deftness) or ideomotor including body part substitution for the tool, timing errors, spatial errors, perseverative or parapraxic errors (i.e., wrong item), or unrecognizable/no response. Although we measured the types of errors associated with both ideomotor apraxia and limb-kinetic apraxia, our analyses in the current article are confined to the former error types. The analyses and interpretation of the limb-kinetic errors will be the subject of a separate article.

Results. The typical (i.e., left) language dominance group ($n = 74$) consisted of 70 dextrals (i.e., right-handed) and 4 nondextrals (i.e., left-handed or ambidextrous). The atypical language dominance group ($n = 16$) consisted of 9 dextrals and 7 nondextrals. The relative proportion of dextrals and nondextrals was significantly different between the typical and atypical language groups ($\chi^2 = 18.03$, $p \leq 0.00002$). Praxis test times postinjection for left (329 seconds \pm 111) and right (317 seconds \pm 81) IAP did not differ overall ($t = 0.78$, not significant [NS]), nor did the left/right times differ for either the typical ($t = 0.94$, NS) or atypical ($t = -1.52$, NS) group. Further, test times postinjection did not differ between the typical and atypical groups ($t = 1.59$, NS). At the time of praxis testing, patients typically had a mild contralateral pronator drift and mild dysphasia (e.g., difficulty with repetition of more complex phrases).

The means (\pm SD) of the praxis scores as a function of injection side, hand, and group are depicted in table 1. Significant findings included a main effect for IAP injection side [$F(1,88) = 5.49$, $p \leq 0.02$] and an interaction for IAP injection side \times language group [$F(1,88) = 12.13$, $p \leq 0.001$]. Follow-up analyses for the IAP injection side \times language group interaction showed that praxis was more impaired for the typical (8.7) than atypical (11.5) language group during left IAP [$F(1,88) = 7.70$, $p \leq 0.007$] but was more impaired for the atypical (10.9) than typical (11.9) language group during right IAP [$F(1,88) = 7.23$, $p \leq 0.009$]. The separate analysis of the typical left language group alone revealed poorer praxis scores during left than right IAP [$F(1,73) = 44.22$, $p \leq 0.0001$] but no difference between the left and right hands [$F(1,73) = 1.47$, NS].

The types of praxis errors in patients with typical and atypical language dominance are depicted in tables 2 and 3. Overall, total errors were significantly more common for the typical (6.9/patient) than the atypical (3.2/patient) language dominance groups [$\chi^2 = 6.13$, $p \leq 0.01$]. Patients with typical language dominance had a greater percentage of ideomotor apraxic errors during left (96%) than right IAP. In contrast, patients with atypical language dominance had a greater percentage of ideomotor apraxic errors during right (69%) than left IAP.

Discussion. We found that the hemispheric lateralization of the temporal and spatial components of skilled movements (i.e., ideomotor praxis) is more closely associated with language dominance than hand preference. As a group, patients with typical left cerebral language dominance also have left cerebral dominance for the temporal-spatial components

Table 1 Mean ($\pm SD$) praxis scores for left and right hands following left and right intracarotid amobarbital procedure (IAP), as function of cerebral lateralization of language (note maximum best-possible score for each hand in each condition is 12)

Group	n	Hand	Left IAP	Right IAP
Typical dominance (left language dominance)	74	Left	8.6 (4.0)	11.8 (0.9)
		Right	8.7 (4.1)	11.9 (0.4)
Atypical dominance (mixed or right language dominance)	16	Left	11.6 (0.9)	10.8 (3.0)
		Right	11.4 (1.1)	10.9 (3.0)
Nondextrals with left language dominance (subset of left language dominance group)	4	Left	7.8 (3.3)	11.6 (0.9)
		Right	8.6 (3.4)	11.4 (0.9)
Dextrals with atypical language dominance (subset of atypical dominance group)	8	Left	11.2 (1.2)	11.4 (0.9)
		Right	11.2 (1.2)	11.4 (1.1)

of skilled motor movements. In contrast, patients with atypical language dominance have more ideomotor apraxic errors during right than left cerebral dysfunction, although the asymmetry in praxis function between the two cerebral hemispheres was less than the asymmetry in patients with typical language dominance. Further, patients with atypical language dominance have overall fewer ideomotor apraxic errors compared to patients with typical language dominance. We propose that apraxic errors are lower in patients with atypical language dominance because their temporal-spatial movement representations are more bilaterally distributed; when one hemisphere is incapacitated, the other hemi-

sphere can compensate. Thus, patients with atypical language dominance appear to exhibit a more bilateral distribution of both language and praxis functions.

The reason that there is a close anatomic relationship between the neural systems that mediate praxis and language functions is unknown. However, this relationship should not be surprising given that language output, whether by speech or manual means, requires highly skilled movements. Corballis¹¹ has suggested that human speech and language did not evolve from nonhuman primate vocalizations but rather evolved from manual gestures. If speech and language evolved from gestures, this may help explain why language and praxis appear to be medi-

Table 2 Comparison of types of praxis errors during left versus right intracarotid amobarbital procedure (IAP) in the left and right hands of patients with typical left language dominance (n = 74)

Side of IAP	Left	Left	Right	Right
Hand	Left	Right	Left	Right
Body part substitution	17	14	4	5
Timing	18	15	3	1
Spatial	21	21	3	1
Perseverative or wrong item	37	32	0	0
No response or unrecognizable	156	162	3	0
Total praxis errors	249	244	13	7
% total errors	48.5%	47.6%	2.5%	1.4%

Table 3 Comparison of types of praxis errors during left versus right intracarotid amobarbital procedure (IAP) in the left and right hands of patients with atypical language dominance (n = 16)

Side of IAP	Left	Left	Right	Right
Hand	Left	Right	Left	Right
Body part substitution	1	1	3	3
Timing	1	1	0	0
Spatial	1	3	0	0
Perseverative or wrong item	4	4	3	3
No response or unrecognizable	0	0	12	12
Total praxis errors	7	9	18	18
% total errors	13.5%	17.3%	34.6%	34.6%

ated by shared or anatomically proximal neural systems for the programming of skilled movements.

Patients with intractable epilepsy are at increased risk of having anomalous hemispheric specialization. Thus, one might question whether our findings are applicable to the general population. However, our data are consistent with prior clinical studies data showing that apraxia is associated with aphasia and that apraxia occurs most frequently after left cerebral damage in dextrals.^{1,4,6,12,13} In clinical studies, approximately 57% to 72% of patients with aphasia are also apraxic.^{1,4,6,12,13} This association between aphasia and apraxia is stronger for lesions that are more acute and involve a larger portion of the cerebral hemisphere.

The IAP produces an acute but transient lesion, which is maximal initially and resolves progressively over approximately 10 minutes.⁸ The area of dysfunction primarily involves the portion of the brain supplied by branches of the ipsilateral internal carotid artery but, to a lesser degree, may include the contralateral frontal region via arterial crossflow and nonperfused structures (e.g., ipsilateral posterior hippocampus) via diaschisis. Because the effects of intracarotid amobarbital are diffuse, both efferent and afferent systems may be involved.

Dysfunction in either the language dominant or nondominant hemisphere may produce apraxia, but the apraxia is more severe when the language-dominant hemisphere is anesthetized than when the nondominant hemisphere is anesthetized. In the previous report of apraxia during the Wada test, patients were tested immediately after injection of the ultra-short-acting barbiturate methohexitol.⁹ In contrast, our patients received the longer-acting barbiturate amobarbital and were tested after partial resolution of their deficits. Overall, our results for patients with typical language dominance are similar to those reported previously, even though our patients had a less-complete hemispheric dysfunction at the time of praxis assessment. Because our patients were tested after partial resolution of their cerebral dysfunction, their errors coded as "no response" during praxis assessment were unlikely to be caused by confusion or the inability to understand because of global aphasia. Many of these patients could not correctly perform the praxis tasks although they could name the tool or describe its use.

Liepmann⁴ suggested that hand preference was more strongly determined by praxis than language dominance. This hypothesis of Liepmann is supported by observations of apraxia without aphasia in left-handed patients after large right cerebral strokes.^{14,15} Based on Liepmann's hypothesis, one might expect that hand preference would be a better predictor of praxis dominance than would language lateralization. However, our results show that language dominance appears to be a greater determinant of praxis lateralization than does hand preference (see table 1). Nondextrals with left cerebral language dominance had a pattern of praxis scores similar to dextrals with left language domi-

nance, whereas dextrals with atypical language dominance had a pattern similar to nondextrals with atypical language dominance. It is possible that part of the dissociation is because of the problem of defining handedness. We dichotomized the patients based on a standard handedness questionnaire. The determination of cerebral lateralization by the Wada language assessment is likely superior to a handedness questionnaire. Nevertheless, there is a clear dissociation between hand preference and lateralization of ideomotor praxis in some patients.

Therefore, our results do not support Liepmann's postulate that hand preference is determined more by the laterality of what he called the movement formula or temporal-spatial engrams than language dominance.⁴ The movement engrams related to ideomotor praxis are strongly colateralized with language function. Hand preference usually is associated with this lateralization (e.g., 93% of the patients with typical language dominance were dextrals), but hand preference may dissociate from the lateralization of language and ideomotor praxis. Thus, hand preference may be affected by other factors such as motor strength/speed, attentional asymmetries,¹⁶ and social/cultural influences.¹⁷

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Does HHV-8 have a protective role on the development of HIV encephalopathy?

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Article abstract—Objective: To evaluate risk factors for HIV encephalopathy and whether Kaposi's sarcoma (KS) and coinfection with human herpesvirus 8 (HHV-8) protect against this disease in a cohort of HIV seroconverters. **Methods:** Individuals with known dates of HIV seroconversion belonging to different HIV exposure categories (intravenous drug users, homosexual men, heterosexual contacts) were recruited by 17 clinical centers throughout Italy. Antibodies to HHV-8 lytic antigens were detected in a subgroup of participants using an immunofluorescence assay. Risk factors for HIV encephalopathy were evaluated using Cox proportional models. The association between KS or HHV-8 infection and HIV encephalopathy was evaluated using standard statistical techniques. **Results:** During the study period, 485 of the 1,520 participants developed acquired immunodeficiency syndrome, 38 of whom developed HIV encephalopathy. HHV-8 serologic status was determined for 390 participants. Male gender, injecting drug use, and low CD4 T-cell count were associated with HIV encephalopathy; none of the 63 participants with KS developed this disease. The risk of HIV encephalopathy did not differ significantly by HHV-8 serologic status. **Conclusions:** HIV encephalopathy was found to be associated with male gender and intravenous drug use. The risk increased at lower CD4 T-cell counts. Although HIV encephalopathy occurred less frequently in patients with KS, no association with HHV-8 infection was found. **Key words:** HHV-8—HIV encephalopathy.

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Encephalopathy induced by infection with the HIV, or “HIV encephalopathy,” is a specific nosographic entity that was first recognized in 1986.¹ Estimates of the incidence of HIV encephalopathy among persons with AIDS vary among studies.^{1–4} In Italy, according to official AIDS notifications, HIV encephalopathy represents approximately 8% of all AIDS-defining diseases at AIDS diagnosis.

Little is known about the cofactors that may influence the risk of developing HIV encephalopathy. Injecting drug use and older age increase this risk, whereas a reduction in risk has been observed in association with the use of antiretroviral drugs such as zidovudine.^{5–9}

A biological study suggests that coinfection with the recently recognized human herpesvirus 8 (HHV-8), which is associated with Kaposi's sarcoma (KS),¹⁰ may protect against HIV encephalopathy.¹¹ HHV-8 encodes two proteins (v-MIP1 and v-MIP2), which show homology to the CC chemokine family and are functionally active. Specifically, HHV-8 v-MIP2 can block infection with HIV-1 by inhibiting virus entry into CD4⁺ cell lines, which express CCR3, a chemokine receptor for HIV-1 entry into microglia. Consequently, microglial cells might be less prone to HIV

infection, reducing the risk of developing HIV encephalopathy.¹¹ However, other biological studies did not find that CCR3 plays a role as coreceptor for HIV entry into microglia.¹²

Because HHV-8 is found in most KS lesions,^{13,14} KS can be used as an indicator of HHV-8 infection. Two published studies have used KS to assess the protective role of HHV-8 on HIV encephalopathy; these studies show that KS patients have a lower risk of HIV encephalopathy when compared with AIDS patients without KS,^{15,16} although direct information on HHV-8 infection was not available.

To better evaluate the potential protective role of HHV-8 coinfection as indicated by the presence of KS or of HHV-8 antibodies, we analyzed risk factors for HIV encephalopathy in a large cohort of HIV seroconverters.

Methods. Analysis was performed on the cohort of individuals of the Italian Seroconversion Study.^{17,18} Study participants had known dates of HIV seroconversion (defined as the midpoint in time between the last negative result on the HIV serologic test and the first positive test result within 2 years), and they belonged to different exposure categories (intravenous drug users [IDU], homosexual men, heterosexual contacts). For the Italian Seroconver-

* See the Appendix on page 2036 for a listing of members of the Italian HIV-Seroconversion Study.

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sion Study, clinical information was routinely collected from participants, who were advised to undergo clinical and laboratory examinations, including CD4 T-cell counts at least every 6 months. Detailed information on the study appears elsewhere.^{17,18} A subgroup of participants with an available serum specimen were tested for HHV-8 antibodies.

Definition of HIV encephalopathy. To define HIV encephalopathy (also known as "HIV dementia," "AIDS dementia," or "subacute encephalitis from HIV"), we used the diagnostic criteria for diseases indicative of AIDS included in the 1987 revision of the AIDS case definition.¹⁹ HIV encephalopathy was defined as "clinical findings of disabling cognitive and/or motor dysfunction interfering with occupation or activities of daily living, or loss of behavioral developmental milestones affecting a child, progressing over weeks to months, in the absence of a concurrent illness or condition other than HIV infection that could explain the findings. Methods to rule out such concurrent illnesses and conditions must include cerebrospinal fluid examination and either brain imaging (CT or MR) or autopsy."

HHV-8 serology. Antibodies to HHV-8 lytic antigens were detected by using an immunofluorescence assay with body cavity B-cell lymphoma (BCBL-1) cell line (obtained through the AIDS Research and Reference Reagent Program, Division of AIDS, National Institutes of Health, from M. McGrath and D. Ganem). BCBL-1 cells were grown in RPMI 1640 medium with 10% heat-inactivated fetal calf serum, antibiotics (penicillin at 100 U/mL and streptomycin at 100 µg/mL), and 5×10^5 mol/L 2-mercaptoethanol. For the immunofluorescence assay, BCBL-1 cells were treated for 48 hours with phorbol 12-myristate 13-acetate (20 ng/mL). Ten microliters of the cell suspension (4×10^6 cells/mL) were smeared on slides, air-dried at room temperature, and then fixed in methanol/acetone, 1:1 (vol/vol), at -20°C for 10 minutes. Titrations were done by fivefold serial dilutions. An inverse titer of 5 or more was considered positive. All microscopic examinations were evaluated by investigators blinded to the status of the specimens. Quantitative detection of antibodies to Epstein-Barr virus (viral cap antigen) was performed to exclude cross-reactivity with HHV-8 antilytic antibodies. A detailed description and validation of the method have been previously reported.^{20,21}

Statistical analysis. The main endpoint of the study was HIV encephalopathy. HIV encephalopathy-free time was calculated from the date of HIV seroconversion to the first occurrence of the event of interest, or to death, or to the cutoff of the study (December 12, 1997).

Risk factors for HIV encephalopathy were investigated through both univariate analysis (i.e., Kaplan-Meier curves) and multivariate analysis (Cox proportional hazards regression models). Fixed variables (i.e., age at HIV seroconversion, gender, exposure category) and time-dependent variables (i.e., antiretroviral treatment, CD4 cell count) were entered in Cox regression models for estimating the adjusted relative hazard (RH) of HIV encephalopathy for the different possible cofactors. The risk of HIV encephalopathy for a certain level of CD4 T-cell counts, after stratifying by exposure category, also was estimated using Kaplan-Meier curves.

To evaluate the association between KS and HIV encephalopathy, we compared the incidence of HIV encephalopathy among KS patients to that among patients without

KS. The RH of HIV encephalopathy for AIDS patients with or without KS was estimated; the association was evaluated using a 95% CI of the RH and through standard statistical tests (two-tailed Fisher exact test).

To study the association between HHV-8 infection and HIV encephalopathy, some of the collaborating centers recruited a subgroup of individuals for whom at least one serum specimen collected after HIV seroconversion was available. An HHV-8 serologic test was performed on these serum specimens, and Kaplan-Meier curves were used to estimate the cumulative probability of HIV encephalopathy since the time of HIV seroconversion for HHV-8-positive and HHV-8-negative participants. A Cox regression model was applied to analyze the association between HHV-8 positivity and HIV encephalopathy after adjusting for the previously mentioned cofactors.

Results. Overall, 1,520 individuals were enrolled in the study; 1,065 (70%) were men and 455 (30%) women. With respect to exposure category, 54% were IDU, 26% were homosexual men, and 19% were heterosexual contacts; the exposure category could not be determined for the remaining 1%. During the study period, 485 of the 1,520 participants (31.9%) developed AIDS, of whom 38 (7.8%) developed HIV encephalopathy.

Risk factors for HIV encephalopathy. We first investigated the possible role of several demographic, behavioral, and immunologic variables. Male gender and low CD4 T-cell count were associated with HIV encephalopathy at the univariate analysis. After adjusting for possible confounders in a multivariate model, intravenous drug use also became significantly associated with HIV encephalopathy (table 1). The risk of encephalopathy increased dramatically for individuals with CD4 T-cell counts below 150 cells/mm³ (data not shown); IDU tended to develop HIV encephalopathy more rapidly than individuals belonging to the other exposure categories.

HIV encephalopathy and KS. Among the 485 participants who developed AIDS, 38 developed HIV encephalopathy, and 63 developed KS. None of the KS patients developed HIV encephalopathy during the study period (OR = 0.0; 95% CI, 0.0 to 0.80); this protective association was statistically significant ($p = 0.01$). In 12 of the 38 patients who developed HIV encephalopathy, this was the only AIDS-defining disease, whereas the remaining 26 individuals had additional opportunistic diseases.

Because the incidence proportion of KS is known to be much higher among homosexual men compared with other exposure categories, we performed an analysis stratified by exposure category. The number of cases and the cumulative incidence of HIV encephalopathy and KS according to exposure category are shown in table 2. The estimates of the cumulative incidence of HIV encephalopathy and of KS were similar for IDU and heterosexual contacts 7 years after seroconversion, whereas homosexuals presented a slightly lower cumulative incidence of HIV encephalopathy and a markedly higher incidence of KS compared with the other two exposure categories.

HIV encephalopathy and HHV-8 serologic status A serum sample for determining HHV-8 serologic status, collected at a median of 1 year after HIV seroconversion (interquartile range, 0.5 to 2.3 months), was available for 390 participants (26% of the overall cohort). The characteristics of this subgroup were similar to those of the entire

Table 1 Univariate and multivariate analysis of determinants of HIV encephalopathy in a cohort of 1,520 individuals with known HIV seroconversion dates (38 cases of HIV encephalopathy)

Characteristic	Univariate model		Multivariate model*		Multivariate model†	
	Crude RH	95% CI	Adjusted RH	95% CI	Adjusted RH	95% CI
Age at seroconversion‡	1.26	0.86–1.84	1.36	0.86–2.16	1.07	0.66–1.73
Males vs. females	2.69	1.05–6.90	2.40	0.92–6.26	3.06	1.17–8.04
Intravenous drug users vs. sexual contacts§	1.46	0.74–2.91	1.89	0.85–4.22	2.26	1.01–5.06
CD4 cell count¶	1.95	1.57–2.41	—	—	1.76	1.40–2.21
Zidovudine	2.63	1.36–5.11	—	—	0.89	0.39–2.09

* Adjusted for fixed covariates (i.e., demographic variables and HIV-exposure category).

† Adjusted for fixed and time-dependent variables (i.e., CD4 T cell count, zidovudine, and variables not shown in the table, such as HIV-related symptoms, and use of prophylaxis).

‡ Relative hazard (RH) estimated for increments of 10 years.

§ Sexual contact consists of homosexual men and heterosexual contacts.

¶ RH estimated for increments of 100 cells.

cohort except for the exposure category: homosexual men were overrepresented; 46% of these individuals were homosexuals, 38% were IDU, 14.9% were heterosexual contacts, and the exposure category was not determined for the remaining 1.0% (n = 4). One hundred forty-seven individuals had HHV-8 antilytic antibodies (35.9%).

Of the 386 participants with known HHV-8 serologic status and known HIV exposure category, 136 (35.2%) developed AIDS, of whom 12 (3.1% of the entire cohort and 8.8% of AIDS patients) developed HIV encephalopathy (7 IDU, 4 homosexual men, and 1 heterosexual contact); 27 (6.9% of the entire cohort and 19.8% of AIDS patients) developed KS (26 homosexual men and 1 heterosexual contact). With regard to HHV-8 serologic status, HIV encephalopathy occurred in 8 of the 147 HHV-8-positive individuals and in 4 of the 239 HHV-8-negative individuals. The cumulative incidence of HIV encephalopathy was 2.6% (0.0 to 5.7) for HHV-8-positive individuals and 1.5% (0.0 to 3.2) for HHV-8-negative individuals by 7 years after HIV seroconversion (figure); the difference was not statistically significant ($p = 0.84$). At 8 years after HIV seroconversion, the risk of HIV encephalopathy was 4.13% and 3.12%, respectively, for HHV-8-positive and HHV-8-negative individuals. Afterward, the risk increased to 6.5% among HHV-8-negative individuals and remained stable among HHV-8-positive individuals.

To evaluate the role of HHV-8 after controlling for other determinants of HIV encephalopathy, crude and adjusted RRs were calculated (table 3). HHV-8 seropositivity did not significantly influence the risk of HIV encephalopathy: the unadjusted and adjusted RRs of HIV encephalopathy were slightly under the unity. Although the power of this analysis was smaller, the other RRs remained similar to

those estimated on the entire cohort. In particular, IDU had a higher RH of HIV encephalopathy compared with individuals belonging to the other exposure categories, with the risk increasing when adjusting for other covariates.

Discussion. Our data, derived from a cohort of HIV seroconverters, confirm that the risk of developing HIV encephalopathy in individuals who develop KS is lower than that in those who do not develop this disease. These data are consistent with the hypothesis of Boshoff et al., who suggest that HHV-8 may inhibit HIV entry into the microglia through CCR3 receptors.¹¹ However, these data should be interpreted with caution, given that this effect may be modified by the dramatic differences in the incidence proportions of KS and HIV encephalopathy in the different exposure categories: KS is likely to occur among homosexual men, whereas HIV encephalopathy is more likely to occur among IDU. The excess of KS among homosexual men can, for the most part, be explained by the higher prevalence of HHV-8 infection in this group, whereas the greater risk of HIV encephalopathy among IDU could result from the influence of drug abuse on cognitive functions²² and from misdiagnosis.

To verify whether the inverse association between KS and HIV encephalopathy is attributable to a protective role of HHV-8, we investigated the association between HHV-8 serologic status and HIV encephalopathy. HHV-8 was first detected in KS lesions,¹⁰ and longitudinal studies suggest its causal

Table 2 Cumulative incidence (risk) of HIV encephalopathy and Kaposi's sarcoma (KS) by exposure category among 1,520 individuals

Characteristic	Cases of HIV encephalopathy, n (%)	HIV encephalopathy risk at 7 years from seroconversion, risk (95% CI)	Cases of KS, n (%)	KS risk at 7 y from seroconversion, risk (95% CI)
IV drug user	26 (3.2)	2.4 (1.2–3.6)	9 (1.1)	0.9 (0.2–1.7)
Homosexual	7 (1.8)	1.9 (0.4–3.6)	49 (12.8)	10.7 (7.2–14.3)
Heterosexual	5 (1.7)	2.7 (0.3–5.2)	4 (1.3)	1.7 (0.0–3.8)

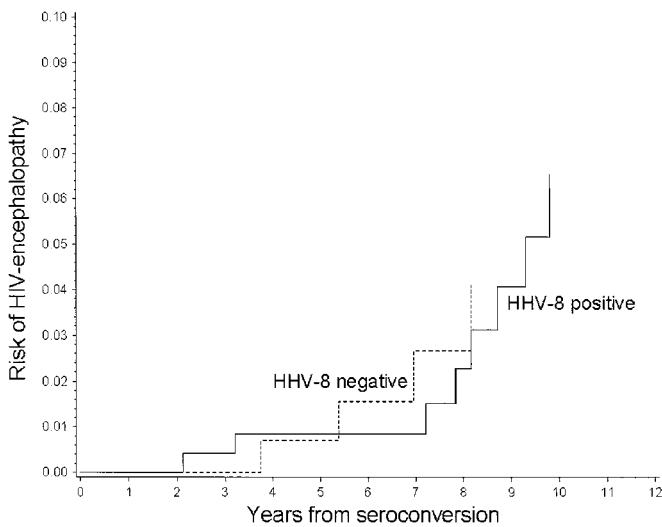


Figure. Kaplan-Meier estimates of the risk of developing HIV encephalopathy among 390 individuals stratified by HHV-8 serologic status.

role in the pathogenesis of KS.^{23,24} In a study conducted on the same group of participants as those in the current study, we found that the risk of developing KS was 14.8 times higher among individuals with anti-HHV-8 lytic antibodies than among HHV-8-negative participants.²⁵ In the current study, we failed to find a significant difference in the risk of HIV encephalopathy between HHV-8-negative and coinfecting individuals. Similar results were obtained by a preliminary analysis of a study conducted on AIDS patients,²⁶ showing an inverse association between HIV encephalopathy and KS but no association with HHV-8 infection. However, the geometric mean titer of HHV-8 antibodies was higher among AIDS patients without HIV encephalopathy compared with those with the disease. The authors therefore suggest that active viral replication, which is associated with the secretion of chemokine-like products, and not HHV-8 infection per se, may pre-

vent the occurrence of HIV encephalopathy. This hypothesis may be explained, at least in part, by the results of studies conducted on AIDS patients. For example, in an unpublished study, we found that the median antibody titer was much higher (1:400) among AIDS patients with KS compared with those with other AIDS-defining diseases (1:25), suggesting that the level of viral activity may greatly differ between KS and non-KS HHV-8-positive individuals.

However, the hypothesis of a competition between HHV-8 and HIV at the brain level remains controversial, as suggested by the conflicting findings of different studies. A recently published study suggests that CCR5 is the predominant coreceptor for infection of human adult microglia by the HIV type 1 encephalopathy isolates and that anti-CCR5 antibodies are able to dramatically inhibit microglial infection by HIV. On the other hand, microglia express lower levels of CCR3, and anti-CCR3 antibodies have little or no effect on infection.¹² These findings contradict the hypothesis of an important protective role of vMIP-II, the chemokine-like product secreted by HHV-8, as well as of other possible agents that may compete for the CCR3 receptors. Nevertheless, vMIP-II also binds the CCR5 coreceptor, although less efficiently, suggesting that this viral product may inhibit HIV entry and replication in microglia also through this pathway.

With regard to the other risk factors, intravenous drug use and male gender were found to be associated with HIV encephalopathy, and risk tended to increase with decreasing CD4 T-cell counts. The RH for the use of zidovudine tended to decrease below unity after adjusting for clinical and immunologic variables, but the association did not reach statistical significance. This lack of significance could result from the observational design of the study, which, compared with clinical trials, is less capable of detecting the effects of antiviral drugs.

Before drawing firm conclusions, some limits and

Table 3 Univariate and multivariate analysis of determinants of HIV encephalopathy in 390 individuals with known HIV seroconversion dates and known HHV-8 serologic status (12 cases of HIV dementia)

Characteristic	Univariate model		Multivariate model*		Multivariate model†	
	Crude RH	95% CI	Adjusted RH	95% CI	Adjusted RH	95% CI
Age at seroconversion‡	1.32	0.70–2.49	1.64	0.74–3.63	1.31	0.54–3.19
Males vs. females	3.63	0.47–28.16	3.64	0.45–29.61	6.79	0.70–65.89
Intravenous drug users vs. sexual contacts§	1.86	0.59–5.90	3.44	0.77–15.41	6.13	1.10–34.25
HHV-8 positive vs. negative	0.89	0.27–2.96	1.01	0.26–3.89	1.00	0.26–3.79
CD4 cell count¶	1.48	1.09–2.02	—	—	1.25	1.01–2.27
Zidovudine	3.16	0.98–10.20	—	—	1.61	0.32–8.12

* Adjusted for fixed covariates (i.e., demographic variables and HIV-exposure category).

† Adjusted for fixed and time-dependent variables (i.e., CD4 T cell count, zidovudine, and variables not shown in the table, such as other antiretroviral drugs, HIV-related symptoms, and use of prophylaxis).

‡ Relative hazard (RH) estimated for increments of 10 years.

§ Sexual contact consists of homosexual men and heterosexual contacts.

¶ RH estimated for decreases of 100 cells.

biases of our study need to be mentioned. First, HHV-8 serology was not available for all study participants. Nevertheless, the sera were provided by a subgroup of the study sites without any selection of the patients to exclude a selection bias. Second, other definitions have been proposed that might better respond to clinical research needs.²⁷⁻²⁹ However, the quality of the centers included in the study was high, most of them having participated in previous studies on HIV encephalopathy where the same definition was used.⁵ Furthermore, the analyses repeated after stratifying by clinical center to control for intercenter variability showed similar results (data not shown).

Our study suggests that HIV encephalopathy is associated with intravenous drug use and low CD4 T-cell count. Although we confirmed the inverse association between HIV encephalopathy and KS, no association with HHV-8 infection was found. Therefore, the results of studies that suggest a protective role of HHV-8 infection on HIV encephalopathy should be cautiously interpreted.

Appendix

Besides the authors, the Italian HIV-Seroconversion Study includes: A. Sinicco (Torino), G. Angarano (Bari), G. Tarantini (Milan), A. Lazzarin (Milan), B. Salassa (Torino), R. Pristerà (Bolzano), U. Tirelli (Aviano), G. Mazzarello (Genova), V. Colangeli (Bologna), F. Castelli (Brescia), F. Alberici (Piacenza), M.A. Ursitti (Reggio Emilia), M. Barbanera (Livorno), M. Zaccarelli (Rome), L. Ortona (Rome), F. Aiuti (Rome).

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Glucose and [¹¹C]flumazenil positron emission tomography abnormalities of thalamic nuclei in temporal lobe epilepsy

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Article abstract—Objectives: To analyze interictal patterns of thalamic nuclei glucose metabolism and benzodiazepine receptor binding in patients with medically intractable temporal lobe epilepsy (TLE) using high-resolution 2-deoxy-2-[¹⁸F]fluoro-D-glucose (FDG) and [¹¹C]flumazenil (FMZ) PET. **Background:** Structural and glucose metabolic abnormalities of the thalamus are considered important in the pathophysiology of TLE. The differential involvement of various thalamic nuclei in humans is not known. **Methods:** Twelve patients with TLE underwent volumetric MRI, FDG and FMZ PET, and prolonged video-EEG monitoring. Normalized values and asymmetries of glucose metabolism and FMZ binding were obtained in three thalamic regions (dorsomedial nucleus [DMN], pulvinar, and lateral thalamus [LAT]) defined on MRI and copied to coregistered, partial-volume-corrected FDG and FMZ PET images. Hippocampal and amygdaloid FMZ binding asymmetries and thalamic volumes also were measured. **Results:** The DMN showed significantly lower glucose metabolism and FMZ binding on the side of the epileptic focus. The LAT showed bilateral hypermetabolism and increased FMZ binding. There was a significant correlation between the FMZ binding asymmetries of the DMN and amygdala. The PET abnormalities were associated with a significant volume loss of the thalamus ipsilateral to the seizure focus. **Conclusions:** Decreased [¹¹C]flumazenil (FMZ) binding and glucose metabolism of the dorsomedial nucleus (DMN) are common and have strong lateralization value for the seizure focus in human temporal lobe epilepsy. Decreased benzodiazepine receptor binding can be due to neuronal loss, as suggested by volume loss, but also may indicate impaired γ -aminobutyric acid (GABA)ergic transmission in the DMN, which has strong reciprocal connections with other parts of the limbic system. Increased glucose metabolism and FMZ binding in the lateral thalamus could represent an upregulation of GABA-mediated inhibitory circuits. **Key words:** Epilepsy—PET—Thalamus—Glucose metabolism—Flumazenil—Volumetry.

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Most patients with medically intractable temporal lobe epilepsy (TLE) have hippocampal sclerosis.¹ Hippocampal sclerosis is associated with hippocampal atrophy as measured by volumetric MRI,² altered glucose metabolism as demonstrated by PET,³ and decreased benzodiazepine receptor binding as shown by [¹¹C]flumazenil (FMZ) PET.^{3–5} The involvement of several subcortical structures, particularly the thalamus, in the pathophysiology of TLE also has been demonstrated. In rodent models of medial TLE, thalamic hypermetabolism is seen during seizures and is associated with seizure spread beyond temporal structures.^{6,7} In humans, the high prevalence of interictal metabolic abnormalities^{3,8} and volume loss⁹ of the thalamus ipsilateral to the seizure support its involvement in TLE.

The dorsomedial nucleus (DMN) is a central part of the network supporting limbic seizure propagation from the primary focus to other cortical and subcortical brain structures.^{6,10–13} The DMN undergoes major neuropathologic changes in animal models of

limbic epilepsy^{14,15} and in humans with prolonged hemiconvulsions.¹⁶ Furthermore, the activation of several other thalamic nuclei has been demonstrated during status epilepticus induced by amygdala stimulation in rats,⁷ in which local blood flow changes¹² and seizure-associated neuropathologic abnormalities¹⁵ have been reported in different regions of the thalamus.

Animal studies suggest that functionally different parts of the human thalamus might be differentially affected in patients with TLE. Because altered γ -aminobutyric acid (GABA)/benzodiazepine receptor function is a prominent feature of the epileptogenic hippocampus and neocortex, we hypothesized that abnormal benzodiazepine receptor binding may occur in regions of the thalamus affected by the epileptic process. Recent developments in functional neuroimaging allow us to analyze alterations of glucose metabolism or receptor binding in small brain structures (e.g., hippocampus) by combining high-resolution PET with MRI coregistration and correction for partial-

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Table 1 Clinical data, MRI, and PET findings of 12 patients with temporal lobe epilepsy

Patient no./sex	Age, y	Seizure type	Seizure duration, y	Focus side	MRI findings	Visual PET findings		Abnormal thalamic AI		
						Hypometabolism	Decreased FMZ binding	MRI	FDG	FMZ
1/F	36	SP, CP	13	R	R HA R T hemangioma	R T, R TH	R T	—	—	D
2/M	18	CP, GTC	14	L	L HA, AA L T ganglioglioma	L > R T	L T	—	—	—
3/M	12	SP, CP	4	R	R HA	R T	R T	—	—	D
4/M	35	SP, CP, GTC	29	L	L HA, AA	L + R T, L TH	L T	—	—	—
5/F	21	SP, CP, GTC	13	R	Normal	R T, F, P, O	R T	—	L	D
6/F	42	SP, CP	23	R	R HA, AA	R > L T, R F-P, R TH	R T	+	D, L, P	D
7/F	41	SP, CP	37	R	R HA	R T, R + L F, O, R TH	R T, F, R + L O	+	D, P	D, P
8/M	47	SP, CP	5	L	L HA, AA	L T, L TH	L T	+	—	—
9/F	24	CP, GTC	23	R	R HA, AA R T atrophy	R > L T, R F-P	R T	+	—	D, P
10/F	53	CP, GTC	40	L	L HA, AA	L T, P, L + R F, L TH	L T, L TH	—	D, P	D, P
11/M	28	SP, CP, GTC	24	L	L HA	L T	L T	+	—	—
12/M	38	CP, GTC	25	L	L HA L T cyst	L T	L T	+	P	—

FMZ = $[^{11}\text{C}]$ flumazenil; FDG = 2-deoxy-2- $[^{18}\text{F}]$ fluoro-D-glucose; SP = simplex partial seizures; CP = complex partial seizures; GTC = generalized tonic-clonic seizures; R = right; L = left; F = frontal; T = temporal; P = parietal; O = occipital; TH = thalamus; HA/AA = hippocampus/amygdala atrophy (by volumetric MRI); D = dorsomedial nucleus; L = lateral thalamus; P = pulvinar; AI = asymmetry index (considered to be abnormal when >2.5 SD above control mean).

volume effects.¹⁷ Using this methodology, we analyzed changes of glucose metabolism as well as benzodiazepine receptor binding in different regions of the thalamus, including the DMN, pulvinar, and lateral thalamic nuclei (LAT), in patients with unilateral TLE.

Methods. Patients and controls. Twelve patients (6 men and 6 women, mean age 32.9 ± 12.4 years) with medically intractable TLE were included in the study (table 1). All patients underwent prolonged video-EEG recordings with scalp/sphenoidal electrodes. Ictal recordings were obtained in 11 patients, whereas only interictal recordings were available in 1 (Patient 10). MRI-based hippocampal and amygdala volumetry was performed in all cases using a previously described method.^{2,18} All patients underwent 2-deoxy-2- $[^{18}\text{F}]$ fluoro-D-glucose (FDG) as well as FMZ PET examination. Antiepileptic treatment at the time of the PET examinations included monotherapy or polytherapy with phenytoin ($n = 5$), carbamazepine ($n = 5$), lamotrigine ($n = 6$), gabapentin ($n = 3$), vigabatrin ($n = 1$), and valproate ($n = 2$).

The control group for defining the normal values for normalized glucose metabolic rates and metabolic asymmetries for the thalamic regions on FDG PET included six right-handed healthy volunteers (five women and one man, mean age 27.5 years, age range 23 to 39 years). The control

group for FMZ PET also included six healthy volunteers (four men and two women, mean age 40.1 years, age range 30 to 50 years). This control group was used to define the normal values for thalamus volume as well as for normalized FMZ binding and FMZ binding asymmetries for hippocampus, amygdala, and thalamic regions. The control subjects were not taking any medication and had no history of neurologic or psychiatric disorder. All of them had normal MRI scans.

MRI procedure. MRI studies were performed on a GE 1.5-T Signa 5.4 unit (GE Medical Systems, Milwaukee, WI). Volumetric imaging was performed using a spoiled gradient-echo (SPGR) sequence. The three-dimensional SPGR sequence generates 124 contiguous 1.5-mm sections of the entire head using a 35/5/1 (TR/TE/NEX) pulse sequence, flip angle of 35 degrees, matrix size of 256×256 , and field of view of 240 mm. These images were obtained in the coronal plane, and the imaging time for this sequence was 9.5 minutes.

PET scanning protocol. All FMZ PET studies were performed in accordance with policies of the Wayne State University Institutional Review Board, whereas FDG PET studies were performed for clinical indications as part of the presurgical evaluation. PET studies were performed using the CTI/Siemens EXACT/HR (Knoxville, TN) whole-body positron tomograph located at Children's Hospital of

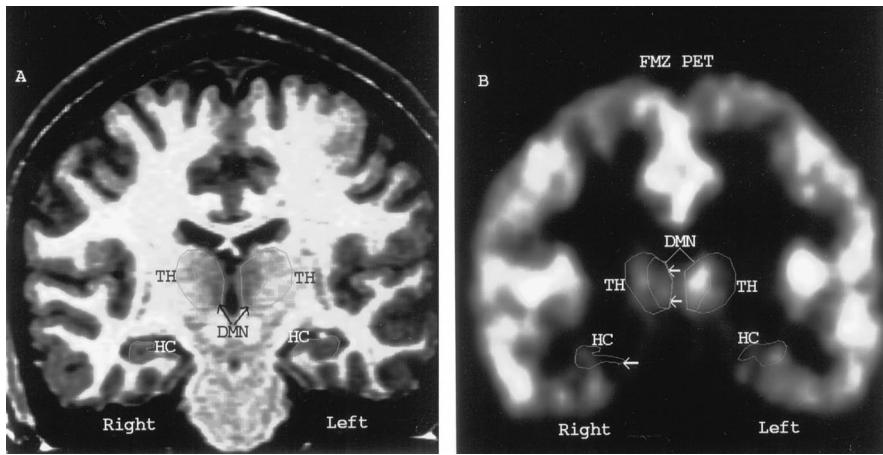


Figure 1. Regions of interest (ROIs) drawn on the MRI using contrast adjustment for the thalamus (TH), dorsomedial nucleus (DMN), and hippocampus (HC) in a patient (no. 1) with right hippocampal atrophy (A). These ROIs were superimposed on the corresponding coregistered [^{11}C]-flumazenil (FMZ) PET image (B). The figure shows a prominent binding asymmetry in the HC and DMN, with lower binding on the right side (thick arrows).

Michigan, Detroit. This scanner has a 15-cm field of view and generates 47 image planes with a slice thickness of 3.125 mm. The reconstructed image in-plane resolution obtained is 6.5 ± 0.35 mm at full-width-at-half-maximum (FWHM) and 7.0 ± 0.53 mm in the axial direction for the FMZ PET, and 5.5 ± 0.35 mm at FWHM and 6.0 ± 0.49 mm in the axial direction for the FDG PET (reconstruction parameters: Shepp-Logan filter with 1.1 cycles/cm cutoff frequency and Hanning filter with 0.20 cycles/pixel cutoff frequency). For FMZ PET, attenuation correction was performed on all images using data from a 15-minute transmission scan of the head. For FDG PET, computed attenuation correction was applied according to the method of Bergstrom et al.¹⁹

Patients fasted for 4 hours before PET studies. Surface EEG electrodes were placed according to the International 10-20 system, and EEG was monitored throughout the PET examinations. A venous line was established for injection of FDG (0.143 mCi/kg) or FMZ (0.4 mCi/kg as a slow bolus over 2 minutes using a Harvard pump) produced using a Siemens RDS-11 cyclotron (Knoxville, TN). External stimuli were minimized by dimming the lights and discouraging interaction so that studies reflected the resting awake state. All patients had their PET performed in the interictal state. For the FMZ PET, a 60-minute dynamic PET scan of the brain was performed (sequence: 4×30 seconds, 3×60 seconds, 2×150 seconds, 2×300 seconds, 4×600 seconds), beginning at the time of injection. Summed images representing activity concentration between 10 and 20 minutes were used to display benzodiazepine receptor binding in brain. For the FDG PET, a 20-minutes static emission scan was initiated 40 minutes after tracer injection.

MRI/PET coregistration and partial-volume correction of PET images. Matching of PET and MR image volumes was performed as previously described,¹⁷ using a multipurpose three-dimensional registration technique (MPItool) developed by the Max-Planck-Institute (Cologne, Germany).²⁰ After converting MRI data to PET data format, the PET image volume was coregistered with the MR image volume using MPItool, and a new image volume was created with image planes corresponding to the original MR image planes. The coregistered PET and MR image volumes were transferred to an SGI OCTANE (Silicon Graphics, Mountain View, CA) workstation, and partial volume

correction of the PET images was performed using an established method.¹⁷

Regions of interest. Brain regions of interest (ROIs) for the hippocampus and amygdala were manually defined on the MRI according to a protocol described previously.^{2,18} ROIs were drawn for left and right hippocampus and amygdala in all planes clearly showing these structures.

In addition, ROIs for the thalamus and thalamic regions were drawn on the MRI. The whole thalamus was defined as the portion of the diencephalon located superior to the interventricular foramen and the hypothalamic sulcus. The landmarks for this region consisted of the plane of the interventricular foramen anteriorly, the wall of the third ventricle medially, the inferior margin of the central part of the lateral ventricle superiorly, the lateral margin of the thalamic gray matter laterally, the posterior margin of the pulvinar posteriorly, and the interventricular foramen, hypothalamic sulcus, and midbrain structures inferiorly. The ROIs for the DMN were drawn following the border of the whole-thalamus ROI (figure 1A). The initial section used to define the anterior boundary of the DMN was the third or fourth section posterior to the interventricular foramen, thus allowing 3 to 4.5 mm of the anterior thalamus for the anterior nucleus of the thalamus. Thereafter, the outline of the lateral margin of the DMN was drawn parallel to the lateral margin of the thalamus at half the distance from the midline to the lateral thalamic border. The posterior boundary of the DMN was defined as the section in which the body of the fornix is formed by the joining of the two crura of the fornix. This was usually between three to five sections (4.5 to 7.5 mm) anterior to the posterior boundary of the pulvinar. The LAT was defined as the area between the lateral margin of the DMN and the lateral margin of the thalamus. The LAT drawn this way included the ventral anterior, ventral lateral, ventral posterolateral, and ventral posteromedial thalamic nuclei.

Mean hemispheric activity concentration values were obtained by drawing hemispheric ROIs (including gray and white matter) directly on the partial-volume-corrected PET images to calculate normalized glucose metabolic rates as well as normalized FMZ binding for the thalamic regions. In patients with TLE, the hemisphere contralateral to the epileptic focus was used for normalization. All

ROIs of all patients and control subjects were drawn by the same person (C.J.).

Thalamus volumetric measurement. Once the ROIs for the whole thalamus had been defined, the volumes were calculated by multiplying the number of voxels in the ROI by the voxel volume to give a total volume of thalamus in cubic millimeters. The absolute volumes, thus obtained, were then "normalized" by correcting them for individual variation in head size. Each volume was presented as the ratio of the thalamic volume to the total intracranial volume (in percent).

For the assessment of thalamic volume asymmetries we used an asymmetry index (AI), that was calculated as follows:

$$AI(\%) = (V_C - V_I)/[(V_C + V_I)/2] \times 100(\%) \quad (1)$$

where V_C and V_I are the volumes (in cubic millimeters) of the thalamus contralateral and ipsilateral to the seizure focus, respectively.

PET image analysis. Visual assessment of PET images. Visual evaluation of FDG and FMZ PET images was performed by three of the authors independently.

Quantitative analysis of the PET images. All ROIs defined on MRIs were copied to the coregistered, partial-volume-corrected FDG and FMZ PET images (figure 1B), and a weighted average concentration for each structure (thalamic regions on both FDG and FMZ PET as well as hippocampus and amygdala on FMZ PET only) was obtained. Values of normalized glucose metabolic rate and FMZ binding were obtained for each thalamic structure by dividing the mean activity concentration values for a given region by the hemispheric activity concentration. For the assessment of asymmetries in glucose metabolism and FMZ binding, we used an AI similar to that calculated for the thalamic volumetric asymmetry measurements:

$$AI(\%) = (A_C - A_I)/[(A_C + A_I)/2] \times 100(\%) \quad (2)$$

where A_I and A_C are the radioactivity concentrations (in microcuries per milliliter) for the defined structures on the side of the focus and contralateral to the focus, respectively.

Study design and statistical analysis. Statistical analysis was performed using StatView statistical package (BrainPower, Inc., Calabasas, CA). Initially, we tested whether significant differences could be detected between left and right thalamic volumes, normalized glucose metabolic rates, and FMZ binding in the control group using Student's *t*-test for paired comparisons. If no significant differences were found for a specific variable, the left and right values were pooled; otherwise, left and right values were analyzed separately. The whole thalamus and the three thalamic regions in each hemisphere obtained from the patient group were separated into those regions ipsilateral and those contralateral to the seizure focus. These two sets of regions were then compared with the corresponding thalamic regions obtained from the control group.

Group comparisons of mean thalamic volumes, normalized glucose metabolic rates, and FMZ binding between the three sets of regions were performed using analysis of variance. Furthermore, the left/right asymmetries for the aforementioned variables were tested using Student's *t*-test for paired comparisons separately within each group.

The normal AI limits were established for the hippocampus, amygdala, and thalamic regions, computed as 2.5 SD above the mean AI value obtained in the control group. To determine if regional asymmetries of the measured variables were abnormal in the individual patients, the AI values were compared with the previously established normal AI limit. Multiple regression was used to assess a possible correlation between age or epilepsy duration (independent variables) and the normalized MRI thalamic volumes, glucose metabolic rates, and FMZ binding of the thalamic regions, as well as the corresponding AIs for these variables (dependent variables). Similarly, assuming that glucose metabolic rates and MRI volumes are not independent, their thalamic AIs were compared to thalamic FMZ binding AIs for possible correlations using multiple regression. In addition, to assess a possible relationship between mediotemporal and thalamic FMZ binding abnormalities, regression coefficients were obtained for FMZ binding AIs derived from amygdala and hippocampus (independent variables) and the three thalamic regions (dependent variables). The significance of each correlation was assessed using the *F*-test. A *p* value less than 0.05 was considered significant after correction for multiple comparisons by using Bonferroni's method.

Results. Clinical data, MRI, EEG, and PET findings of the 12 patients are shown in table 1. Unilateral hippocampal atrophy was found in all but one patient (Patient 5; normal hippocampal volumes) and amygdala atrophy was found in 6 patients. In addition, 4 patients had lesions on MRI in the temporal lobe in addition to hippocampal atrophy, ipsilateral to the seizure focus. Visual PET evaluations showed decreased temporal glucose metabolism and temporal FMZ binding (including mediotemporal structures) ipsilateral to the EEG focus in all patients. Mediotemporal seizure onset could be seen in all patients with ictal EEG ($n = 11$). There was no correlation between age or epilepsy duration and the normalized volumes, glucose metabolic rates, or FMZ binding, as well as AIs, in any analyzed region.

Thalamus volumetric findings. Mean normalized volumes of the right and left thalami in the control subjects ($n = 6$) were not statistically different ($0.362\% \pm 0.019\%$ and $0.362\% \pm 0.016\%$, respectively), which allowed us to pool data from right and left to obtain a mean value. The normalized volumes of the thalami ipsilateral to the seizure focus of patients with TLE ($0.327\% \pm 0.033\%$) proved to be significantly lower than the mean volume of the control subjects ($p = 0.028$). Comparison of normalized volumes of the contralateral thalami ($0.354\% \pm 0.041\%$) did not differ significantly from the control mean volume ($p = 0.57$). Paired comparison showed significantly smaller volumes on the side of the focus compared with the contralateral side ($p = 0.0002$).

The mean AI for the normalized volumes of the thalami in control subjects was $2.32\% \pm 1.76\%$, and thus the upper limit of normal volume asymmetry (mean \pm 2.5 SD) was 6.72% . Six of 12 patients had abnormally high AIs, always with smaller volumes on the side of the focus (table 1).

Thalamic glucose metabolism. In the control group, the normalized glucose metabolic rates of the right and left DMN and LAT were not significantly different. Thus, left and right values were averaged for comparison with the patient group. In contrast, the normalized glucose meta-

Table 2 Mean \pm SD normalized glucose metabolic values and asymmetries for the thalamic regions of 12 patients with temporal lobe epilepsy versus control subjects

Region	Control subjects	Patients		
		Ipsilateral to the focus	Contralateral to the focus	p Value (paired <i>t</i> -test)
Dorsomedial nucleus	1.218 \pm 0.093	1.183 \pm 0.093	1.225 \pm 0.080	0.047
Lateral thalamus	1.054 \pm 0.104	1.142* \pm 0.066	1.170* \pm 0.060	0.16
Pulvinar†	1.085 \pm 0.074	1.005 \pm 0.167	1.020 \pm 0.113	0.58

* Mean value is significantly different from control mean values (analysis of variance).

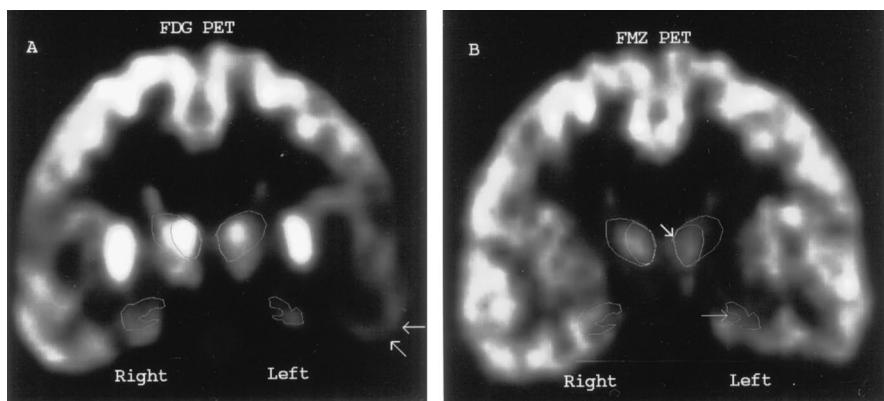
† Pulvinar showed significant left > right metabolic asymmetry in control subjects.

bolic rate of the left pulvinar was significantly higher than that of the right pulvinar for control subjects ($p = 0.005$); therefore, we did not combine these values but used them separately to compare them with the corresponding values of patients with right ($n = 6$) and left ($n = 6$) epileptic foci. Normalized glucose metabolic rates for patients with TLE were significantly higher in the LAT bilaterally ($p = 0.01$ and $p = 0.001$ ipsilateral and contralateral to the focus, respectively) compared with the control subjects. The normalized glucose metabolic values of the other thalamic regions (DMN and pulvinar) were not significantly different from those of the control subjects. Paired comparisons showed significant metabolic asymmetry only in the DMN, with lower glucose metabolism on the side of the epileptic focus ($p = 0.047$).

In the control subjects, the upper limit of the AI was 6.3% for the DMN, 10.5% for the LAT, and 10.2% for the pulvinar. We found a high variability in the AI for the LAT, although there was no left/right bias. Abnormal thalamic AIs of glucose metabolism with lower values on the

side of the seizure focus occurred in at least one thalamic region in 5 of the 12 patients (table 2, figure 2A). An abnormally high AI with lower glucose metabolism contralateral to the seizure focus occurred in the pulvinar of one patient (no. 12).

$[^{11}\text{C}]$ Flumazenil binding in thalamus. In the control group, normalized FMZ binding in the right and left DMN, LAT, and pulvinar was not significantly different. Therefore, we again combined the values for the right and left regions, and the combined means were compared with the corresponding values determined from the patients. The mean FMZ binding of the DMN ipsilateral to the epileptic focus was significantly lower in the patients compared with control subjects ($p = 0.045$; table 3). In contrast, the mean FMZ binding of the LAT contralateral to the focus was significantly higher than in the control group ($p = 0.031$), whereas FMZ binding in the ipsilateral LAT showed a tendency toward increase ($p = 0.059$). FMZ binding in other thalamic regions (pulvinar ipsilateral and contralateral to the focus, as well as the DMN contralat-



normal asymmetry in the hippocampus (thin arrow; AI: 21.4%), the amygdala (thick arrow; AI: 7.5%), with lower values on the left side.

Figure 2. 2-Deoxy-2- $[^{18}\text{F}]$ fluoro-D-glucose (FDG) and $[^{11}\text{C}]$ -flumazenil (FMZ) PET images of Patient 10, who had marked left hippocampal and mild left amygdaloid atrophy. FDG PET (A) shows hypometabolism in the left lateral temporal cortex (arrows), whereas the hippocampal abnormality was less pronounced. An abnormal asymmetry of glucose metabolism was detected in the dorsomedial thalamic nucleus (DMN; asymmetry index [AI]: 16.0%; lower on the left). FMZ PET (B) showed ab-

Table 3 Mean \pm SD normalized $[^{11}\text{C}]$ flumazenil binding and asymmetries for the thalamic regions of 12 patients with temporal lobe epilepsy versus control subjects

Region	Control subjects	Patients		
		Ipsilateral to the focus	Contralateral to the focus	p Value (paired <i>t</i> -test)
Dorsomedial nucleus	1.02 \pm 0.05	0.959* \pm 0.068	1.039 \pm 0.087	0.0018
Lateral thalamus	0.828 \pm 0.055	0.886 \pm 0.075	0.895* \pm 0.084	0.33
Pulvinar	0.847 \pm 0.11	0.807 \pm 0.125	0.819 \pm 0.093	0.63

* Mean value is significantly different from control mean values (analysis of variance).

eral to the focus) did not differ significantly from the control values (all p values > 0.43). Paired comparisons showed that FMZ binding in the DMN was significantly lower on the side of the epileptic focus compared with the corresponding contralateral values ($p = 0.0018$).

The upper limit of the FMZ binding AI in the control group was 6.6% for the DMN, 11.3% for the LAT, and 11.3% for the pulvinar. AIs in the DMN were abnormally high in seven patients (table 1, figure 2B), including 3 patients with abnormal pulvinar asymmetry (the lower values appeared on the side of the focus in all of these cases).

There was a significant correlation between the AIs of the DMN and amygdala ($r = 0.77$, $p = 0.009$), but not between those of the amygdala and other thalamic regions ($p = 0.95$ and 0.09 for the LAT and pulvinar, respectively). There was no correlation between the FMZ binding AI in hippocampus and thalamus ($p = 0.14$, 0.88, 0.58 for the DMN, LAT and pulvinar, respectively) as well as between that in hippocampus and amygdala ($p = 0.17$). There was no correlation between volumetric or glucose metabolic AIs and FMZ binding AIs of any thalamic regions (p values between 0.23 and 0.83).

Discussion. This study demonstrates localized abnormalities of glucose metabolism and benzodiazepine receptor binding in the thalamus in patients with TLE. Our findings show that the FMZ binding decrease is especially common and prominent in the DMN and has a strong lateralization value for the seizure focus. It can be associated with ipsilateral thalamic volume loss, suggesting that neuronal loss can be a factor contributing to this abnormality. However, the effect of volume loss should be small in our PET measurements because partial volume correction was performed. Because the DMN has dense reciprocal connections with the ipsilateral amygdala as well as with other parts of the limbic system,^{11,21} our findings suggest that the DMN plays an important role in the pathophysiology of human TLE, particularly in the propagation of epileptic discharges. The asymmetric glucose metabolism of the thalamus is consistent with previous findings.^{3,8} Increased glucose metabolism and FMZ binding in the LAT could represent an upregulation of the GABA-mediated inhibitory circuits in this part of the thalamus.

Methodologic considerations. A number of methodologic issues in our study should be addressed. Our patient group included people with medically intractable TLE who had concordant EEG, MRI, and PET evidence of unilateral temporal lobe seizure foci. Although extratemporal neocortical FDG ($n = 5$) and FMZ ($n = 1$) PET abnormalities were seen in some patients on visual PET evaluation, it is well known that such alterations can be found commonly in mesial TLE³ and may represent remote effects rather than primary epileptogenic areas. Although four patients also had temporal lesions on MRI in addition to hippocampal atrophy, these were associated with ipsilateral hippocampal atrophy as well as mesiobasal seizure onset on ictal EEG. It is known that mesial and lateral temporal areas have dense,

reciprocal connections with each other, and ictal discharges commonly propagate from one to the other. Thus, the individual contributions of these portions of the temporal lobe on the extratemporal functional abnormalities cannot be distinguished. Therefore, we believe that despite the relatively wide range of PET and MRI abnormalities of our patients, they represent a reasonably homogeneous population for evaluating the effect of TLE on thalamic glucose metabolism and benzodiazepine receptor binding.

The thalamic ROIs were drawn on high-resolution MRI using a protocol we believe is reliable for outlining the borders of the three thalamic regions, including the DMN. The signal intensity of this major thalamic nucleus is also different from the surrounding areas on the MRI (figure 1). Both the region of the pulvinar and the LAT include several smaller nuclei that could not be identified separately. The anterior thalamus could be drawn typically in only two or three MRI planes and showed very low activity on PET images, with high interindividual variability. Thus, although we included this region in the volumetric measurement, we excluded it from PET analysis.

Visual PET evaluation was done on non-partial-volume-corrected images and failed to find thalamic asymmetries in several cases. For FMZ PET, the low thalamic activity made it even more difficult to assess localized asymmetries; this may explain the low sensitivity of visual evaluation of thalamic abnormalities (table 1). These findings further emphasize the necessity of partial volume correction in the analysis of small brain structures. The volume of thalamic regions (usually ranging between 1 and 2 cm^3) is comparable with that of the hippocampal subregions that have been identified reliably in previous PET studies.¹⁷ The low interindividual variabilities resulting from the use of normalized glucose metabolic rates and FMZ binding instead of absolute measures were favorable for group comparisons. We used mean hemispheric activity values for normalization obtained from the hemisphere contralateral to the focus because it would be less likely to be affected by localized metabolic or benzodiazepine receptor changes.

Glucose metabolism was higher in the left compared with the right pulvinar in all six normal subjects. The defined ROIs in this region might also include the lateral posterior nucleus, and this metabolic asymmetry might be attributed to the previously reported structural and functional asymmetry of the human posterior thalamus with left-sided dominance.²² This is thought to be caused by the asymmetric pattern of cortical connections in this thalamic region, which is probably related to the language specialization of the dominant thalamus.²² Thus, we accepted this normal asymmetry and did not combine the left and right metabolic values for group comparisons, but used them separately for statistical comparison with the pulvinar of patients with left- and right-sided TLE foci.

Our patients were taking anticonvulsant medication, some of which probably decreased global brain glucose metabolism.^{23,24} The use of normalized metabolic values, however, diminished this effect. It is also unlikely that general metabolic changes selectively affected some parts of the thalamus while leaving other regions unaffected. The metabolic asymmetries also cannot be attributed to drug effects. Thus, it is reasonable to assume that our findings reflect disease-related abnormalities rather than drug effects.

PET abnormalities in the dorsomedial nucleus of the thalamus. One of the main findings in the current study is the demonstration of localized decreased FMZ binding in the DMN ipsilateral to the seizure focus in patients with TLE. This asymmetry has a strong lateralization value for the seizure focus and can be useful for clinical purposes: its presence may verify the side of the epileptic focus.

Altered benzodiazepine receptor binding in the thalamus has been reported previously in patients with generalized epilepsy²⁵ and in TLE.³ Our findings show that decreased FMZ binding is common and can be especially prominent in the DMN. This abnormality was not detected using statistical parametric mapping,⁴ but recent studies using ROIs and correction for partial-volume effects proved that benzodiazepine binding abnormalities are not confined to the mesiotemporal structures in TLE associated with hippocampal atrophy.¹⁷ The finding of thalamic volume loss is consistent with the recent findings of DeCarli et al.,²⁴ who demonstrated thalamic atrophy in patients with complex partial seizures of left temporal origin. Although loss of GABA receptor-containing cells can be a potential cause of decreased benzodiazepine receptors in the DMN, the lack of correlation between the degree of thalamic volume loss and decreased FMZ binding suggests that these PET abnormalities cannot be explained simply by neuronal loss.

Our findings confirm that the thalamus, and especially the DMN, may play an important role in human TLE, one that probably involves propagation pathways and regulation of the spread of epileptic discharges.²⁶ This is consistent with findings from animal studies showing that the blockade of excitatory transmission in the DMN blocks limbic motor seizures induced by pilocarpine.²⁷ Furthermore, the amygdala is important for ipsilateral seizure propagation in the limbic system, including the DMN,¹² and the correlation between amygdala and DMN FMZ binding abnormalities suggests that these structures interact strongly in human TLE. Although benzodiazepine receptor binding abnormalities in the DMN may be primary changes supporting seizure propagation, altered benzodiazepine receptor density can also be a response to recurrent abnormal electrical activity. In support of the second possibility, animal models of limbic seizures demonstrated that prolonged seizures can cause progressive pathologic changes in the DMN,^{14,15} similar to the

thalamic changes in patients dying after prolonged status epilepticus.¹⁶

The thalamic glucose metabolic asymmetry is consistent with findings from several previous studies showing lower glucose metabolism in the thalamus ipsilateral to the epileptic focus.^{3,28} A very recent study²⁹ showed that hippocampal cell loss causing decreased efferent neuronal activity correlates with thalamus hypometabolism in TLE, thus supporting a primary role for mesiotemporal structures in functional changes of the thalamus. Although this correlation was seen bilaterally, our results show that thalamic asymmetry of glucose metabolism can be more widespread than the FMZ binding abnormality and can affect any part of the thalamus. Nevertheless, our findings strongly support the pivotal position of the DMN in the limbic seizure network of human TLE.

Increased [¹¹C]flumazenil binding and metabolism in the lateral thalamus. Increased glucose metabolism and FMZ binding was confined to the LAT. Although widespread hypermetabolism can occur during seizures or in the postictal period,³⁰ all of our PET studies were performed in the interictal state as verified by EEG, and opposite changes in other thalamic regions (see earlier) also suggested that these increases could not be attributed to transient ictal or postictal alterations. Although the increased metabolism and FMZ binding in the LAT was an unexpected finding, previous studies using various animal models of epilepsy have shown that repeated seizures can induce an increased number of benzodiazepine receptor binding sites in different parts of the brain. For example, increased GABA/benzodiazepine receptor number in dentate granule cells of the hippocampus³¹ after repeated seizures is associated with a long-lasting augmentation of messenger RNAs of different GABA(A) receptor subunits.³² An increased density of γ -hydroxybutyrate binding sites was also reported in the lateral thalamus in an animal model of absence seizures.³³ The lateral thalamus in humans consists of several functionally different nuclei, including the thalamic relay nuclei, and it appears to be preferentially involved in the generation of spike and wave discharges.³⁴ This thalamic region is also a part of an intrathalamic GABAergic inhibitory circuit that modulates the strength of afferent input into the thalamus.³⁵ In animal studies, both complex partial and generalized convulsive seizures could be elicited by injecting carbachol into the lateral but not into the medial thalamus.³⁶ In another study, amygdala kindling resulted in signs of long-term membrane remodeling that continued even 2 weeks postseizure in the lateral dorsal thalamus,³⁷ suggesting long-lasting changes in brain morphology and chemistry in this part of the brain of kindled animals. Although these changes were unilateral, it was suggested that contralateral changes may occur at later stages of kindling, as had been demonstrated with regard to *c-fos* expression during evolution of kindling.³⁸ In patients with TLE, it can

be hypothesized that the concomitant FMZ binding increase and hypermetabolism of the LAT indicate enhanced synaptic activity due to an upregulated GABAergic system. These increases could represent an activated inhibitory circuit as a response to the epileptic process, but also a compensatory receptor upregulation due to the progressive loss of afferent thalamic GABAergic input. The existence of such compensation was suggested by previous studies showing interictal hypermetabolism in the temporal lobe contralateral to the seizure focus.³⁹ Moreover, Franceschi et al.⁴⁰ reported bilateral interictal hypermetabolism in the cortex and in subcortical structures in drug-naïve patients with TLE. A recent study also demonstrated that remodeling of neuronal circuits can spread beyond the sclerotic hippocampus and is related to the duration of epilepsy.⁴¹

Our findings provide *in vivo* evidence that different thalamic nuclei undergo different functional alterations in human TLE. These changes are especially important when considering the role of the thalamus in regulation of cortical excitability and seizure propagation. Whether these patterns of abnormal glucose metabolism and benzodiazepine receptor binding in the thalamus are specific for TLE or also are present in extratemporal epilepsies requires further study.

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Postictal *in situ* MRS brain lactate in the rat kindling model

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Article abstract—Objective: To determine the temporal and spatial extent of the lactate (Lact) changes as correlated with seizure characteristics and EEG changes in the rat kindling model. **Background:** Prior studies using MRS have detected cerebral Lact postictally in animal models of seizures and in patients with intractable focal epilepsy. **Methods:** We performed MRS in sham control rats ($n = 4$) and in rats stimulated in the right hippocampus at two different stages of the kindling and at three time points after the seizures: <2 hours ($n = 8$ and 5, stage 0 and stage 5), 2 to 3 hours ($n = 5$ and 6), and >3 hours ($n = 4$ and 2). Lact/creatinine (Cr) and *N*-acetylaspartate (NAA)/Cr ratios were measured in six contiguous voxels (three left, three right) covering the hippocampi, anterior and posterior regions, and compared with EEG and ictal behavior. Lact/Cr ratios were measured at a very low level in the sham control rats and in the >3-hour group. **Results:** In the <2-hour group, Lact/Cr increase was higher in stage-5 rats as compared with stage-0 rats ($p = 0.001$, unpaired *t*-test) and sham control rats when all the voxels were considered. Lact/Cr ratios were higher in the stimulated area as compared with all other brain areas in stage-0 rats ($p = 0.05$, paired *t*-test) but not in the stage-5 rats. Similar results with more inter-animal variability were measured in the 2- to 3-hour group. NAA/Cr ratios increased significantly after stage-0 kindling in the stimulated hippocampus but not after stage-5 kindling. **Conclusions:** Postictal Lact increase as assayed by MRS correlates with EEG and behavioral seizures and suggests that it would be an additional noninvasive technique for seizure localization during the presurgical evaluation of patients with intractable focal epilepsy. **Key words:** Lactate—Epilepsy—Magnetic resonance spectroscopy—Kindling—Chemical shift imaging.

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MRS is a noninvasive technique that has been recently used for the *in situ* studying of metabolic changes in patients with medically intractable partial epilepsy. Various interictal and early postictal metabolic changes have been described.¹ Interictal *N*-acetylaspartate (NAA) has been shown to be decreased in patients with chronic mesial temporal lobe epilepsy with hippocampal sclerosis and cell loss.^{2–6} An ictal and early postictal increase in lactate has been shown in MRS of animal models of generalized epilepsy or status epilepticus.^{7–10} Analogous to what is described in EEG¹¹ and SPECT¹² studies, the

postictal MRS studies may add valuable localizing information. Recent human observations are promising.¹³ The finding of focal lactate increase within the epileptogenic region for several hours after a seizure suggests that postictal MRS may be a useful adjunctive test for the localization of epileptogenic foci. The role of MRS in postictal localization of seizures and its correlation with both EEG onset and spread are not known. The chemical shift imaging (CSI) technique permits the simultaneous acquisition of MRS spectra from various areas of the rat brain and the sampling of relatively small voxels.

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Table 1 Temporal lactate increases after stage-0 and stage-5 kindling

Group	Stage 0, voxel 4	Stage 0, voxels 1, 2, 3, 5, 6	Stage 5, voxel 4	Stage 5, voxels 1, 2, 3, 5, 6
Sham control	0.03 ± 0.03*† (n = 4)	0.05 ± 0.01 (n = 4)	—	—
Immediate <2 h	0.23 ± 0.04* (n = 8)	0.13 ± 0.03 (n = 8)	0.39 ± 0.04† (n = 5)	0.35 ± 0.03† (n = 5)
Intermediate 2–3 h	0.17 ± 0.05‡ (n = 5)	0.08 ± 0.03‡ (n = 5)	0.21 ± 0.07§ (n = 6)	0.14 ± 0.06§ (n = 6)
Delayed >3 h	0 (n = 4)	0.02 ± 0.01 (n = 4)	0 (n = 2)	0.01 ± 0.01 (n = 2)

Time of scanning represents the elapsed time between the end of the stimulation and the beginning of MRS scanning.

* Comparison between sham control and stage 0, immediate scanning, voxel 4: $p = 0.05$.

† Comparison between sham control and stage 5, immediate scanning, voxels 4 and 1, 2, 3, 5, 6: $p = 0.001$.

‡ Comparison between voxel 4 and voxels 1, 2, 3, 5, 6 after stage-0 kindling, intermediate scanning: $p = 0.04$.

§ Comparison between voxel 4 and voxels 1, 2, 3, 5, 6 after stage-5 kindling, intermediate scanning: $p = 0.03$, unpaired t -test.

The kindling model of seizures provides a well-known model of focal seizures and propagation of seizures.^{14–17} The repeated administration of an initially subconvulsive electrical stimulus results in progressive intensification of seizure activity, leading to a generalized convulsion. In rats stimulated in the limbic system, the initial stimulus often elicits focal electrical seizure activity (after-discharge as recorded on EEG) without overt clinical seizure activity (class 0).^{18,19} Subsequent stimulations lead to an intensification of behavioral seizures, which usually evolve through the following classes: 1) facial clonus, 2) head nodding, 3) forelimb clonus, 4) rearing, and 5) rearing and falling.^{18,19} In addition, the after-discharges become longer and more diffuse in the brain. The seizures in the first classes mimic those found in human partial limbic seizures; the seizures in later classes are consistent with limbic seizures evolving into generalized convulsive seizures.¹⁶

We studied the *in situ* spatial and temporal postictal metabolic changes in lactate as assayed by CS¹-proton MRS imaging in kindled rat brains. These changes are directly correlated with the various patterns of EEG ictal onset and spreading as well as behavioral manifestations of kindled rats.

Methods. *Animal treatment.* Male Sprague-Dawley rats (n = 65) purchased from Charles River Laboratories (MA), weighing 200 to 250 grams, were used. The animals were housed under standard laboratory conditions conforming to institutional policies and maintained under a 12-hour light/dark cycle with free access to food and water. Under pentobarbital anesthesia (40 mg/kg IP), each rat was implanted with two single-contact electrodes secured in the skull over the right frontal and left parietal cortex. A bipolar electrode made of two twisted enameled stainless steel wires (Medwire, Mt. Vernon, NY) with vertical inter-electrode distance 0.5-mm was implanted unilaterally in the right hippocampus (anterior +2.5 mm, lateral +4.5 mm, ventral -5 mm). The coordinates (in mm) were calculated with respect to lambda. All electrodes were connected to a microconnector and embedded in dental cement (Temp Bond NE, Kerr Manufacturing Company, Romulus, MI).

Animal kindling protocol. A week after the right hippocampal electrode implantation, the threshold of stimulation was defined for each animal. The electrical stimulation consisted of a burst of monophasic square

waves of 1 msec duration at 60 Hz, for a total duration of 2 seconds. The initial current intensity of 50 μ A was increased by 50- μ A steps every 2 minutes until an after-discharge was recorded. Only animals with stimulation intensity thresholds between 300 and 500 μ A were included in the study. Animals with a threshold >500 μ A or with a motor ictal component directly were excluded. The threshold intensity, stable for a given animal, was then delivered twice a day. As previously described, postdischarge duration was measured from the beginning of the electrical stimulation to the last recorded spike.¹⁹ Differences in spike frequencies or spike amplitudes were not considered. The number of stimulations needed to elicit stage-5 seizures varied between 13 and 15 stimulations per animal. The animals were declared kindled after three stage-5 seizures.¹⁹ A 24-hour delay or more was respected before the last stimulation and the prescanning stimulation. Because of the lack of significant differences between the various stimulation parameters used, there were no correlations made between the MRS abnormalities and both the threshold intensities and number of stimulations needed to elicit stage 5.

MR spectroscopic acquisitions. The rats were scanned at different time points of the kindling process: sham control group (rats were implanted in the right hippocampus but never stimulated); stage-0 group (rats were scanned after a stage-0 seizure); and stage-5 group (rats were scanned after a stage-5 seizure). The elapsed time between the end of the stimulation and the beginning of the scanning was variable (table 1).

After stimulation, animals were watched for behavioral seizures. Two-channel EEG recordings from the left fronto-parietal area and the right hippocampal formation were performed in all animals before and after the electrical stimulation.

Rats were then anesthetized with 2% isoflurane (Abbott Laboratories, Abbott Park, IL) in a mixture of 30% oxygen and 70% nitrous oxide immediately and at various time points after the triggered seizure. After orotracheal intubation with a polyethylene tube (PE-205), the rats were mechanically ventilated (Harvard rodent ventilator, model 683, Harvard Apparatus, South Natick, MA) with 1.5% isoflurane without the use of muscle relaxant. The cortical electrodes and the connector were then removed.

Arterial blood gas level (pH, PCO_2 , and PO_2) was controlled after realization of the scanning except in the stage-5 >3-hour group. Atropine sulfate (0.1 mg) was injected IM to reduce salivation before MRI. The proper body

temperature was maintained throughout the MRI/MRS period by circulating temperature-controlled water underneath the rat platform.

MRS was performed using a 4.7-T CSI-II 40-cm horizontal bore imaging spectrometer (General Electric, Milwaukee, WI), which was equipped with GE Acustar self-shielded gradient coils (maximum 200 mT/m). A revised saddle coil²⁰ with a diameter of 35 mm and window length of 40 mm, which was used for both radio frequency (RF) wave transmitting and signal receiving, was mounted on a plastic cradle. Anesthetized rats were placed in the supine position with head centered in the RF coil. The platform body temperature was maintained by circulating temperature-controlled water underneath the rat platform throughout the MRS/MRI acquisition.

After shimming (i.e., optimizing the magnetic field over the head), a sagittal image, using gradient recalled echo (GRE) pulse sequence through the midline of the brain, was acquired to localize the hippocampal formation. Coronal and transverse GRE images were also acquired. The pituitary gland was used as internal landmark for selecting the volume of interest (VOI) for MRS acquisitions. Using T1-weighted axial images, the areas of scanning were selected. As shown in figure 1, the middle voxels were centered at the hippocampal body level. The electrode hole was not included in the VOI (see figure 1B). The absence of hemorrhage or gross hemispheric damage was systematically checked in all scanned rats. MRS was collected from a 3-mm-thick transverse plane. Using six outer volume suppression pulses to select region of interest (ROI) and three Chess pulses to suppress the water peak, a spin-echo sequence with a 16×16 phase encoding was used to collect all proton MRS data. Field of view of 64 mm and ROI of $8 \times 12 \times 3$ mm³, resulting in a nominal voxel size of $4 \times 4 \times 3$ mm³, was acquired. A total of six spectra were obtained from a rat brain with these parameters. Other acquisition parameters were as follows: repetition time = 3,600 msec and echo time = 135 msec. Lactate signal was inverted as doublet of 7 Hz apart due to J-modulation.²¹ The acquired MRS raw data were transferred to a Sun workstation (Sun Systems, Palo Alto, CA) and processed with a commercial MR software package (SA/GE, General Electric). Before Fourier transform, a 3-Hz line broadening was applied to the free induction decay for signal-to-noise enhancement. All spectra were then curve-fitted to a group of gaussian-shaped peaks using a Marquardt-Levenberg algorithm. Peak areas were used as a relative measure of the corresponding metabolite concentrations (lactate, NAA, and creatine). The creatine peak area was chosen, as an internal reference by assuming the total creatine concentration was not changed.²²

At the end of the experiments, animals were sacrificed after the scanning, and their brains were saved in 10% formalin solution. The localization of the stimulating electrode placement was checked in all animals. Rats with stimulating electrodes positioned outside the hippocampal area were eliminated from the study.

Statistical analyses. Mean \pm standard deviations were calculated for each area(s) of interest for each rat (mean of all voxels, right stimulated hippocampus, all voxels except right hippocampus). Statistical analyses were performed using SPSS (Version 6.1 for Windows, SPSS Inc., Chicago, IL). Comparisons between two different groups were done

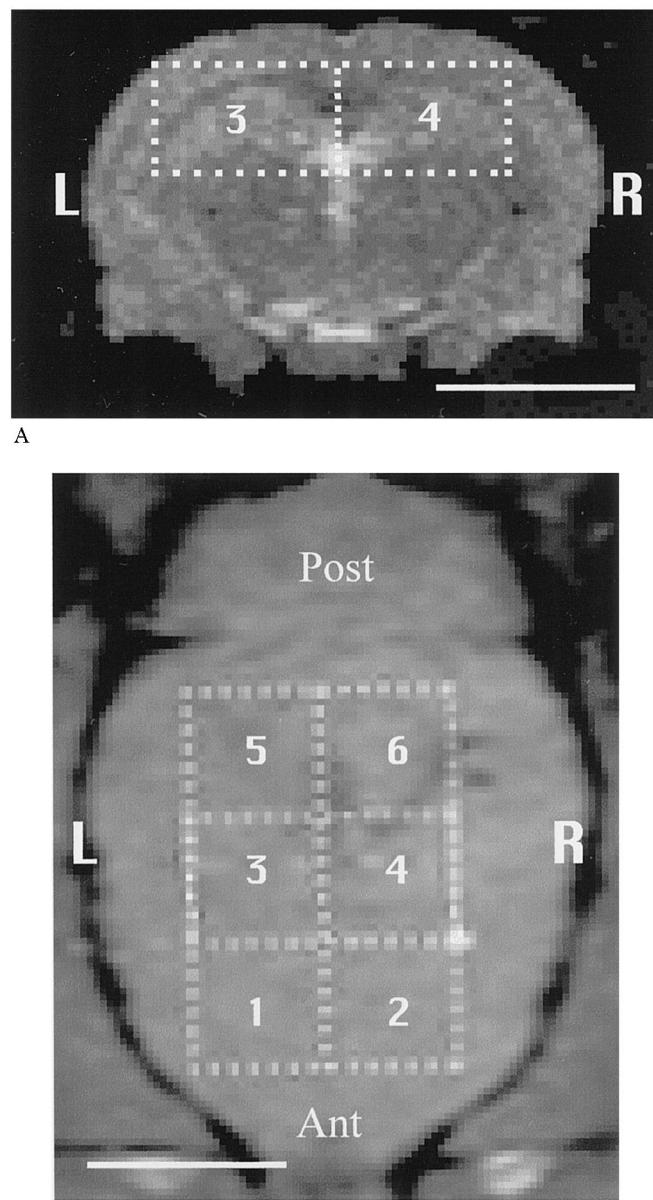


Figure 1. (A) T2-weighted coronal and (B) T1-weighted axial MRS of a rat brain showing the areas studied (the voxels are shown in dotted lines). Scale bar = 5 mm.

by Student's *t*-test for grouped data. Multiple group comparisons were performed using one-way analysis of variance (ANOVA). Comparisons within groups were done using *t*-test for paired data. Significance was taken at $p < 0.01$ and 0.05 levels.

Results. Data from 34 rats were included for the results analyses. Thirty-one rats were eliminated from the study due to inadequate stimulation and/or electrode placement, stimulating/recording intrahippocampal electrode disconnection, or surgical morbidity/mortality. The earliest postictal scanning time point was 90 minutes after the end of the electrical stimulation. Other postictal time points of 2 and 3 hours were used to better define the temporal postictal metabolic changes in lactate ratios.

Electrical and behavioral seizures. As previously described, the repetition of electrical stimulation in the hip-

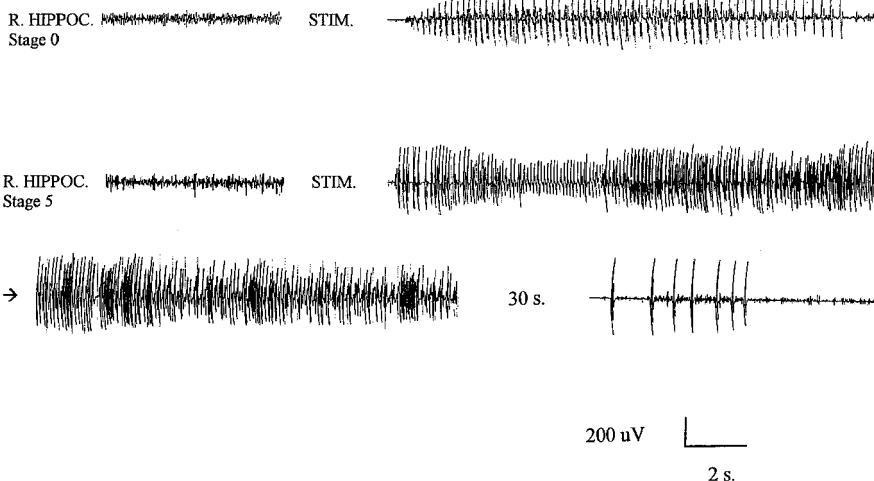


Figure 2. Ictal depth EEG recordings from the right hippocampal area after the first stimulation and during a stage-5 seizure in a kindled rat. Horizontal bar = 2 seconds, vertical bar = 200 μ V.

pocampus was associated with a progressive increase in ictal discharge duration and severity of the behavioral seizure.^{15,19} The first stimulation triggered a short after-discharge but no overt ictal behavioral changes (stage-0 group). Repetition of electrical stimulation triggered a seizure characterized by a longer electrical discharge (figure 2) that was associated with rearing and falling behavior (stage-5 group). Animals were randomly assigned for scanning at various time points after the electrical stimulation. There were no significant differences in the ictal discharge duration between the three scanned groups (early, intermediate, and delayed) after stage-0 kindling (26 ± 9.5 , 19.5 ± 2.5 , and 23 ± 1.5 sec) and after stage-5 kindling (59.5 ± 8 , 57.5 ± 15 , and 60 ± 0 sec) (one-way ANOVA).

Arterial blood gases were checked after scanning in all animals. Animals with arterial blood pH of more than 7.5 and less than 7.3 were eliminated from the study. There were no significant differences in pH and PCO_2 data between the various treatments and scanning groups (one-way ANOVA).

EEG changes. Stage-0 seizures were associated with short-duration and high-amplitude EEG spike discharges (see figure 2). The discharges were consistently predominant on the stimulated side. The ictal EEG discharges were bilateral, symmetric, and longer in stage-5 seizures.

Lactate changes. Figure 3 shows representative postictal brain proton MRS spectra acquired at two different stages of the kindling process. The data were quantified and expressed as ratios of lactate over creatine (figures 4 and 5, and see table 1). Lactate was almost absent in all scanned voxels of sham control animals (including the area of electrode placement).

Immediate (<2 hours) in situ postictal lactate changes. As shown in table 1, after stage-0 seizures, the most pronounced increase in lactate/creatinine ratios was recorded from the middle voxels ($R > L$) in the vicinity of the stimulated right hippocampal area. Moreover, higher lactate ratios were found in voxel 4 (sampling the right hippocampal area of stimulation) as compared with the average ratios from the other nonstimulated areas ($p = 0.05$, $n = 8$, paired t -test). The focal increase in lactate ratios in the area of stimulation corresponded to the location of the ictal EEG pattern as recorded after electrical stimulation. A trend toward a right-sided predominance of lactate increase was noted ($p = 0.33$, $n = 8$, paired t -test). Average

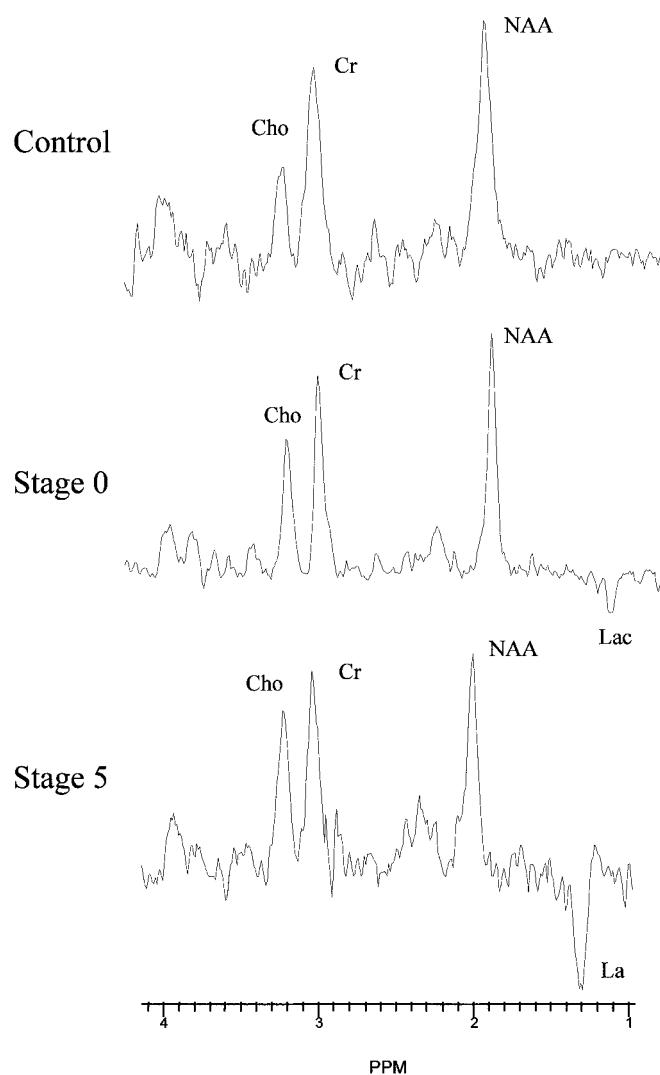


Figure 3. Representative MRS spectra from the right hippocampal area in a sham control animal and in rats scanned immediately (<2 hours) after stage-0 and stage-5 seizures. The 1.3-ppm deflection that corresponds to the lactate is absent in control rat spectra but is present and larger after stage-5 > stage-0 seizures.

Temporal changes in brain lactate after hippocampal kindling

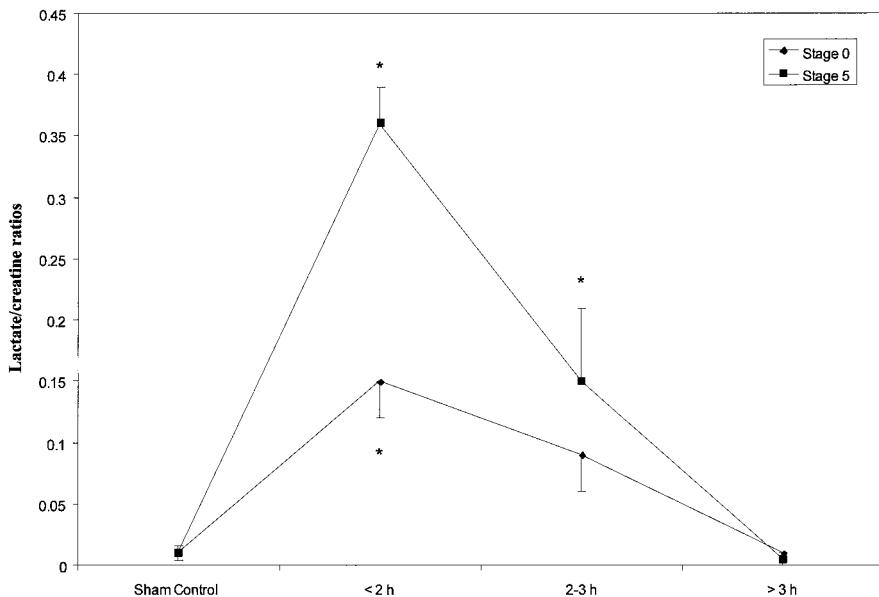


Figure 4. Whole brain lactate ratios (mean \pm standard error of the mean) recorded from various treatment groups. Note the higher lactate ratios after stage-5 seizures and the decrease in lactate ratios during the later postictal stages (>2 hours). *Significant differences in lactate ratios between control values and postictal ratios (significance level of 0.05).

lactate ratios from all scanned voxels were increased in the immediate postictal period after one single stimulation as compared with sham controls ($p = 0.004$, $n = 4$ and 8, unpaired t -test).

Stage-5 seizure triggering was associated with an early postictal whole brain increase in lactate ratios as compared with the ratios recorded from the sham control group ($p < 0.001$, $n = 5/4$, unpaired t -test). The lactate increase was more diffuse after stage-5 seizures (as compared with the more focal pattern found after stage-0 kindling), with no evidence of significant regional/focal predominance (only a trend toward a right-sided and right-hippocampal predominance were noted, $p = 0.52$, $n = 5$, paired t -test). Moreover, there were no differences between right or right + left hippocampal lactate increases and the other scanned areas ($p = 0.07$, and $p = 0.42$, respectively, $n = 5$, paired t -tests). The pattern of lactate increase corre-

sponded to the generalized pattern of ictal EEG activity as assayed by the right and left recording electrodes.

The more severe behavioral convulsions and EEG generalized seizures of stage-5 kindling were associated with significantly higher immediate postictal whole brain lactate ratios as compared with the more focal and less intense seizures of stage-0 kindling ($p = 0.001$, $n = 5/8$, unpaired t -tests). There was no apparent effect of the discharge duration on the lactate ratios within either one of the two kindling groups.

Intermediate (2 to 3 hours) in situ postictal lactate changes. A trend toward similar results was found in rats scanned during the intermediate time point (2 to 3 hours postictal). Averages of whole brain lactate ratios from all scanned voxels (see table 1) decreased in the stage-5 group as compared with the immediate (<2-hours) ratios ($p = 0.02$, $n = 6/5$, unpaired t -test). The decrease

Lactate changes after stage 0 and stage 5 kindlings

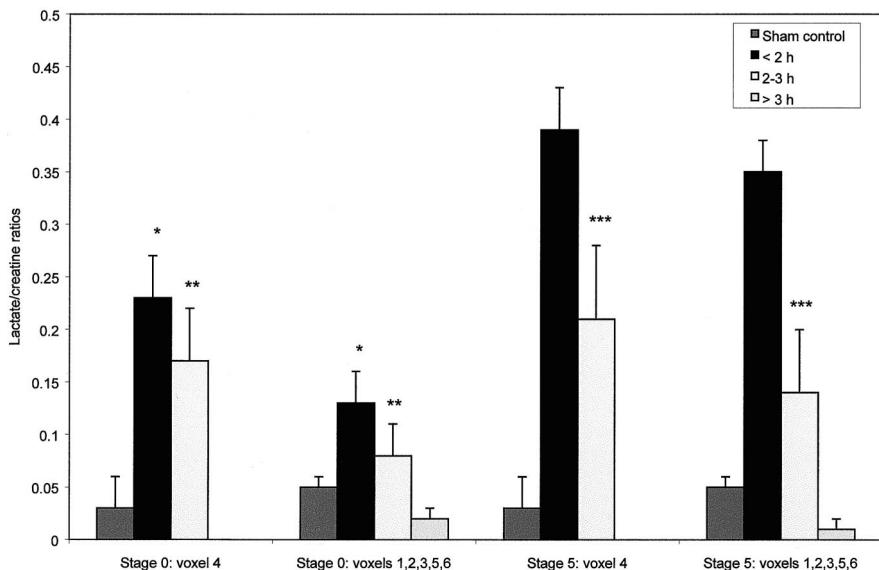


Figure 5. Regional lactate ratios (mean \pm standard error of the mean) recorded from the right stimulated hippocampus (voxel 4) after stage-0- and stage-5-triggered seizures as compared with the lactate ratios from the other scanned area (voxels 1, 2, 3, 5, 6). *, **, ***Significant differences between lactate ratios measured in right hippocampus as compared with all other brain areas (significance level of 0.05).

Table 2 Temporal NAA/creatinine increases after stage-0 and stage-5 kindling

Group	Stage 0, voxel 4	Stage 0, voxels 1, 2, 3, 5, 6	Stage 5, voxel 4	Stage 5, voxels 1, 2, 3, 5, 6
Sham control	1.21 ± 0.09* (n = 4)	1.21 ± 0.15 (n = 4)	—	—
Immediate <2 h	1.34 ± 0.05* (n = 8)	1.21 ± 0.05* (n = 8)	1.25 ± 0.11 (n = 5)	1.25 ± 0.02 (n = 5)
Intermediate 2–3 h	1.24 ± 0.05 (n = 5)	1.14 ± 0.05 (n = 5)	1.26 ± 0.1 (n = 6)	1.23 ± 0.09 (n = 6)
Delayed >3 h	1.26 ± 0.07 (n = 4)	1.13 ± 0.04 (n = 4)	1.14 ± 0.06 (n = 2)	1.03 ± 0.01 (n = 2)

Time of scanning represents the elapsed time between the end of the stimulation and the beginning of MRS.

* Comparisons between sham control and stage 0, immediate scanning, voxel 4, and between voxel 4 and voxels 1, 2, 3, 5, 6 after stage-0 kindling, immediate scanning: $p = 0.04$, unpaired t -test.

NAA = *N*-acetylaspartate.

was not significant after stage-0 seizures (unpaired t -test). These results showed more inter-animal variability even for the same discharge duration and identical behavioral manifestations. The right hippocampal lactate predominance, as compared with the other scanned voxels, was still present in stage 0 ($p = 0.04$, n = 5, paired t -test) and was higher after stage-5 kindling ($p = 0.03$, n = 6, paired t -test). The nonsignificant trend for higher lactate ratios to the right side of the rat brain for both treatment groups was comparable with the immediate postictal results.

Delayed (>3 hours) in situ postictal lactate changes. A decrease in lactate ratios was found in all scanned brain areas during the late postictal period both after stage-0 ($p = 0.05$, n = 4/5, unpaired t -test) and stage-5 ($p = 0.002$, n = 2/6, unpaired t -test) hippocampal kindling groups as compared with earlier postictal stages (see table 1).

NAA/creatinine changes. NAA peaks were identified and quantified. As shown in table 2, NAA/creatinine ratios increased in the area of stimulation (right hippocampal area) as compared with the corresponding area of the sham control group during the immediate postictal period after stage-0 kindling ($p = 0.04$, unpaired t -test). Moreover, early postictal right hippocampal NAA/creatinine ratios in stimulated rats were increased as compared with ratios in the other voxels combined of the electrically stimulated rats ($p = 0.04$, unpaired t -test). There were no regional differences in various VOIs of the sham control brains ($p = 0.9$, paired t -test). A minimal increase in NAA ratios was found in electrically stimulated brains as compared with sham controls ($p = 0.12$, unpaired t -test). NAA/creatinine ratios slightly increased in the right hippocampal voxel and the other scanned areas during the immediate postictal period after stage-5 kindling ($p = 0.96$, not significant, unpaired t -test). There were no significant increases seen during the intermediate and delayed postictal periods.

Discussion. Our study is the first to show a direct *in vivo* correlation between *in situ* lactate increases and the electroclinical patterns in an animal model of focal and secondary generalized seizures. Our *in situ* MRS results showed a significant postictal elevation of lactate ratios both in the electrical stimulation site and in areas of EEG seizure propagation after hippocampal kindling. These changes are short lasting and correlate with the kindling stage. These results confirm our previous findings of lactate increase during the ictal and early postictal phases of seizures. Lactate ratios returned to baseline values

in animals scanned at least 3 hours after stage-0 and stage-5 kindling. We previously showed that lactate increases persist up to 24 hours after kainic acid (KA)-induced status epilepticus.^{10,23} Our previous data suggest that a long-lasting lactate increase (>5 hours) after status epilepticus is associated with the expression of seizure-induced neuronal loss. A significant increase in lactate ratios was observed in KA-treated rats during and 24 hours after seizure onset, and this increase was prevented by cycloheximide (a protein synthesis inhibitor that protects against KA-induced cell damage).²³

Using other models of short-duration cortical electroshock model (<5 minutes), brain lactate levels were shown to decline and reach baseline levels approximately 1 hour after the seizure induction.^{7,8} The mechanisms behind the focal accumulation of lactate in the seizure-onset zone remain unknown. Recently, Petroff et al.²⁴ found that accumulation of lactate after seizures induced by electroshock is due to a temporary equilibrium that was reached between lactate production and clearance. Moreover, the ictal increase in lactate ratios could be the result of a mismatch between glycolysis (which produces lactate) and oxidative metabolism.^{10,25-27} A long-lasting increase that persists well beyond the ictal period (up to 24 hours) may be due to an irreversible failure of the cellular clearance mechanisms that result from prolonged excitotoxicity. It was previously shown that early metabolic impairment may serve as a sensitive index of neurotoxicity because signs of hypermetabolism and substrate depletion precede overt cell degeneration.²⁸⁻³² Thus, the early and prolonged increase in lactic acid in areas expressing neuronal damage could be a reflection of early microscopic damage due to KA neurotoxicity. A less probable mechanism to account for the increase in lactate/creatinine ratios could be due to increase in phosphocreatine or creatine concentrations, or both.

The use of a controlled model of short-duration focal hippocampal seizure and secondary generalized convulsive seizure has enabled us to establish a good correlation between the spatial postictal lactate increases and the ictal electroclinical patterns. In the model of focal hippocampal seizure (stage 0), the lactate increase was higher to the side of the stimulated

hippocampus. The focal lactate increase was higher in all scanned brain areas after the stage-5 seizures (a model of generalized convulsions). The more widespread lactate rise with disappearance of the hippocampal predominance was well correlated with the generalized motor pattern and the longer and more diffuse ictal discharges. In rats scanned after stage-0 seizures, the postictal lactate increase was also seen (though not significant) in contralateral hippocampus and other brain areas. Stage-0 focal seizures were triggered at the lowest current needed to produce focal ictal discharges. However, a variable spread of the discharges to the contralateral hemisphere was recorded from EEG recordings. Previous studies have shown that electrically induced seizures rapidly activate hippocampal pathways bilaterally³³ because of the prominent commissural pathways that are a characteristic of the Sprague-Dawley rat. The propagation of the activation after focal electrical stimulation was correlated with an increase in the c-fos immediate early genes³⁴⁻³⁹ and an increase in ¹⁴C-deoxyglucose metabolism.³⁸ Moreover, partial volume effect³⁹ could contribute to the apparent diffusion of the lactate distribution in stage 0.

A postictal lactate increase that is well correlated with the ictal electroclinical pattern was consistently recorded during the immediate postictal period (<2 hours). More variable changes were seen during later stages after electrical stimulations (2 to 3 hours postictal). After 3 hours, there was no significant lactate increase in any of the scanned areas of stage-0 and stage-5 seizures. These findings are in concordance with previous reports using maximum electroshock model that showed normalization of lactate levels 60 minutes after the seizure induction.^{8,24} Therefore, we suggest that early postictal lactate changes as assayed by MRS may be useful in situ markers for seizure localization and spread in patients with focal epilepsy.

Short-duration focal electrical stimulation led to a transient and focal increase in NAA/creatinine ratios after stage-0 kindling ($p < 0.04$) but not after stage-5 kindling ($p = 0.96$). The NAA/creatinine increases are less pronounced after hippocampal kindling than those found during and after prolonged KA-induced status epilepticus.²³ The increased NAA/creatinine ratios during the ictal phase could be due to increased NAA levels or decreased creatine levels, or both. Because NAA is the main component of NA ¹H-MRS peak, the transient increase in NAA/creatinine ratios during the ictal phase could be due to an increase in NAA synthesis that could result from the activation of a pool of preexisting synthesizing enzymes; it has been previously shown that activity of *N*-acetylated-alpha-linked acidic dipeptidase, an enzyme that hydrolyzes *N*-acetylaspartylglutamate into NAA and glutamate, is elevated in hippocampi from genetically epilepsy-prone rats after seizures.⁴⁰ We speculate that the smaller increase in NAA ratios after hippocampal kindling could be due to the short seizure duration that fails to activate a signifi-

cant pool of preexisting synthesizing enzymes⁴⁰ as compared with KA-induced status epilepticus (1 minute versus 35 hours). Further studies are needed to study the correlations between seizure duration and NAA changes.

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Cognitive correlates of ^1H MRSI-detected hippocampal abnormalities in temporal lobe epilepsy

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Article abstract—*Objectives:* To examine associations between ^1H magnetic resonance spectroscopic imaging (^1H MRSI)—detected hippocampal creatine to N-acetylaspartate (Cr/NAA) ratios and neuropsychological measures sensitive to mesial temporal lobe function. *Background:* The measurement of ^1H MRSI-detected hippocampal metabolites has proved effective in determining extent and lateralization of neuronal damage. However, relationships between ^1H MRSI-detected hippocampal metabolic abnormalities and specific areas of cognitive functioning have received limited attention compared to other studies using MRI volumetry or cerebral blood flow techniques. *Methods:* We analyzed right and left hippocampal Cr/NAA ratios in 46 adult mesial temporal lobe epilepsy patients (32 left, 14 right) by ^1H MRSI at high magnetic field (4.1 T). We examined the relationship between the right and left Cr/NAA hippocampal ratios to measures of verbal and visual memory, intelligence, attention, visuoperception, and confrontation naming. *Results:* Measures of episodic verbal memory ($n = 33$) and visual confrontation naming ($n = 46$) were selectively associated with left hippocampal metabolic function ($p < 0.004$), whereas neuronal function of the right hippocampal region was strongly associated with performance on a measure of facial recognition ($n = 46$; $p < 0.02$). *Conclusions:* This study shows that specific areas of cognitive function are related to hippocampal neuronal metabolic abnormalities as detected by spectroscopic imaging. The current study indicates that ^1H MRSI offers a complimentary technique to structural imaging studies in the study of mesial temporal lobe epilepsy and may enhance understanding of the role of hippocampal function in complex cognitive systems. **Key words:** ^1H MRSI—Mesial temporal lobe epilepsy—Neuropsychology.

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Mesial temporal lobe sclerosis (MTS) is characterized by hippocampal neuronal cell loss and gliosis and is a common feature in patients with mesial temporal lobe epilepsy (MTLE). Such structural ab-

normalities often are associated with both intractable partial complex seizures and neuropsychological deficits, particularly episodic memory functioning. Research incorporating structural (MRI volumetric

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measurement, histopathologic cell counts) and functional MRI (fMRI; PET) neuroimaging techniques has found associations between mesial temporal lobe structures and episodic memory.¹⁻³ Several reports have found that material-specific associations exist between left MTS and verbal memory deficits, whereas generally less-consistent associations have been found between right MTS and nonverbal memory deficits.^{1,4,5} However, studies using preoperative MRI hippocampal volumetric protocols and histopathologic analysis of hippocampal cell counts have not always found these material-specific memory associations.^{6,7} For example, Lencz et al.⁵ found a positive relationship between prose recall performance (Wechsler Memory Scale-Logical Memory % recall) and left hippocampal volume, but found that only total MRI-measured volume of the left temporal lobe related to a task of word list learning. Verbal memory components such as verbal memory span and semantic and temporal order processing appear related to a broader neural network involving temporal and extratemporal neocortical structures.^{8,9} Further studies have suggested that the effects of hippocampal pathology impact context-rich episodic memory (verbal and nonverbal), whereas extrahippocampal areas (including entorhinal, perirhinal, and temporal neocortex) contribute more to context-free semantic memory, attention, and higher level lexicon systems.^{10,11}

Within the past decade, proton magnetic resonance spectroscopic imaging has proved a reliable method to detect and lateralize mesial temporal lobe neuronal loss and glial abnormality found in intractable MTLE.^{12,13} ¹H MRSI can reliably measure N-acetylaspartate (NAA), which reflects neuronal mitochondrial activity and is sensitive to hippocampal neuronal loss.¹⁴ ¹H MRSI shows greater sensitivity in detecting neuronal cell loss than MRI volumetric measurement alone.¹⁵ Recent ¹H MRSI studies have identified an abnormal interictal metabolic profile in patients with MTLE.^{12,13}

Despite advances in ¹H MRSI technology, the extent to which neurobehavioral measures of cognition relate to "functional" neurochemical analyses of mesial temporal lobe brain structures has been limited. Other functional imaging techniques such as PET show activity-related changes in regional cerebral blood flow (rCBF) under certain cognitive demands.^{16,17} However, ¹H MRSI provides a neuroimaging technique through which *in vivo* cell metabolism and intrinsic cell energetics can be recorded, thereby offering a unique window into neuronal functional integrity and neurobehavioral function. Although the clinical utility of ¹H MRSI in detecting epileptic-associated mesial temporal neuronal damage has been established, characterization of the neuropsychological correlates associated with ¹H MRSI-detected abnormalities has only been examined sparsely. In a study investigating ¹H MRSI-detected mesial temporal lobe abnormalities, Rocchetta et al.¹⁸ found that verbal memory performance in pa-

tients having undergone right temporal lobectomy with contralateral metabolic abnormalities performance was similar to that of left temporal lobectomy patients. In a study of adult schizophrenics, ¹H MRSI-measured left mesial temporal lobe creatine levels were found selectively associated to several measures of verbal and visual memory, although frontal creatine levels were not associated with these same cognitive measures.¹⁹

In this report, we examine potential associations between ¹H MRSI-detected neurochemical status of the left and right hippocampus measured at 4.1 T to neuropsychological measures sensitive to hippocampal function. This study sought to establish neuropsychological correlates to spectroscopic imaging of hippocampal regions in patients with MTLE.

Methods. *Subjects.* We studied 46 patients from a consecutive series of 52 patients who underwent ¹H MRSI as part of a preoperative evaluation for possible surgery for intractable MTLE at our center. All patients underwent standard preoperative diagnostic procedures, including video/EEG monitoring, interictal EEG, and imaging studies according to our center protocol as described previously.¹³ Lateralization of seizure onset was left temporal in 32 patients and right temporal in 14 patients. Only those patients who had preoperative neuropsychological evaluation were included in the current study. Preoperative neuropsychological evaluation and spectroscopic imaging were always performed within 3 days of each other. Most patients received the two procedures on the same day. Anterior temporal lobectomy (ATL) was performed on 42 of the 46 patients. All patients who underwent ATL had pathologic confirmation of mesial temporal sclerosis. Patients from this series were excluded if the imaging or pathologic findings showed other coexisting lesions (i.e., tumors, vascular lesions).

Nuclear magnetic resonance studies at 4.1 T. The ¹H MRSI studies were acquired using a 4.1-T whole-body imaging/spectroscopy system and quadrature-driven tunable matchable head coil.¹⁵ All studies were performed before surgical implantation or resection and with approval by the institutional review board. Published details of the ¹H MRSI acquisition protocol are provided elsewhere.^{13,15} The quantitative value for the hippocampus consisted of the ratio of creatine to N-acetylaspartate (Cr/NAA). Higher values of the ratio Cr/NAA reflect abnormal metabolic function (>1.0 , 2 SD). The protocol for obtaining the ¹H MRSI ratios in this sample has been described in detail elsewhere.¹³ Table 1 presents mean Cr/NAA hippocampal ratios for the left and right patient groups. Mean left hippocampal Cr/NAA ratio for both groups was 1.06 with a range of 0.50 to 1.98, whereas mean right hippocampal Cr/NAA ratio for both groups was 1.03 with a range of 0.56 to 1.91. Skewness and kurtosis values reflected generally normal distributions for both left and right ratios. Bilateral asymmetric Cr/NAA elevation was detected in eight left and two right MTLE patients. The Cr/NAA ratio was higher in the seizure focus side for each of these 10 patients. Statistical analysis revealed no differences ($p > 0.05$) between MTLE patients with bilateral Cr/NAA ratios and MTLE patients with unilateral Cr/NAA ratios in

Table 1 Demographic, clinical, and neuropsychological test characteristics in the left and right mesial temporal lobe epilepsy (MTLE) patients

Characteristic	Left MTLE	Right MTLE
Age, y	35.7 (10.2)	33.0 (9.7)
Women/men	20/12	8/6
Education	12.7 (2.1)	11.6 (2.6)
Mean age at seizure onset	11.8 (8.9)	18.7 (13.0)*
Seizure duration, y	24.1 (11.9)	14.1 (10.0)†
Handedness, right/left	31/1	14/0
LH MRS Cr/NAA ratio	1.2 (.34)	0.74 (.24)‡
RH MRS Cr/NAA ratio	0.89 (.39)	1.3 (.43)†
Full-scale IQ	86.1 (9.7)	89.0 (14.7)
Verbal IQ	87.0 (10.0)	89.1 (12.2)
Performance IQ	86.3 (10.1)	90.2 (18.2)
Boston naming test	41.5 (11.4)	46.9 (10.7)
LM immediate	13.4 (6.2)	15.7 (2.0)
LM % recall	50.9 (23.0)	75.6 (11.5)†
VR immediate	9.2 (2.3)	9.8 (3.4)
VR % recall	79.6 (25.7)	86.3 (18.3)
Facial recognition	43.2 (5.5)	42.7 (4.2)

Standard deviations are presented in parentheses.

* $p < 0.05$; † $p < 0.01$; ‡ $p < 0.001$.

MRS = magnetic resonance spectroscopy; Cr/NAA = creatine to N-acetylaspartate; LM = Wechsler Memory Scale logical memory; VR = Wechsler Memory Scale visual reproduction.

terms of age, age at seizure onset, seizure duration, education, IQ, left Cr/NAA ratio, and right Cr/NAA ratio.

Neuropsychological measures. All patients received comprehensive neuropsychological evaluation, including measures of verbal and performance intelligence (VIQ and PIQ from the Wechsler Adult Intelligence Scale-Revised²⁰ [WAIS-R]). Episodic verbal memory testing consisted of the Logical Memory (LM) subtest from the Wechsler Memory Scale (WMS²¹). Also administered was the Boston Naming Test (BNT²²), a 60-item visual confrontation naming test. The BNT also has been described as a measure of semantic memory requiring retrieval of long-term semantic knowledge. Immediate verbal-free recall (LM I) and 30-minute delayed verbal recall (LM II) were measured using LM. Scoring followed the standardized format outlined by Russell.²³ In addition, an LM savings score was calculated ($[\text{LM II/LM I}] \times 100$). Episodic visual memory was assessed using the visual reproduction immediate (VR I) and delayed recall (VR II) portions of the WMS. A VR savings score (VR % recall) was calculated ($[\text{VR II/VR I}] \times 100$). The Benton Test of Facial Recognition (BFRT²⁴) was used as a task of visuoperception and consists of matching a target face to its compliment face among several faces under differing lighting and orientations. This task does not involve a memory component and has been found to be sensitive to inferotemporal lobe damage.^{25,26} The Paced Auditory Serial Additions Test (PASAT²⁷) was used as a measure of sustained auditory attention and typically has not been found sensitive to mesial temporal lobe dysfunction.³

Statistical analysis. A series of *t*-tests were used to examine clinical and demographic differences between the left and right MTLE patients. Nonparametric analyses were used for the handedness and gender distribution differences. Pearson coefficient correlations were calculated examining bivariate relationships between the neuropsychological measures and the Cr/NAA hippocampal ratios. Correlational analyses were performed on the entire patient sample to avoid attenuation of range problems. We included patients with both left and right MTLE so that we would extend the range of MRS hippocampal values. The predictive relationships between the ¹H MRSI measures of hippocampal function and each neuropsychological measure were examined separately using a series of stepwise regression analyses. We entered ¹H MRSI left Cr/NAA hippocampal ratio, right Cr/NAA hippocampal ratio, age at habitual seizure onset, and epilepsy duration (years) in a stepwise fashion using $p < 0.05$ for entrance and $p < 0.10$ for removal. Each of the neuropsychological variables (table 2) was separate dependent variables.

Power analysis was performed because of the large number of correlations and also because of the varying sample sizes among the dependent variables. We set power level to detect a medium effect size (power = 50%). When a correlation involved the VIQ, PIQ, BFRT, PASAT, or BNT, the effect size of $r = 0.28$ was required for significance ($N = 46$, power = 50%, $p < 0.05$). When a correlation involved LM immediate, LM % recall, VR immediate, or VR % recall, the effect size of $r = 0.34$ was required for significance ($N = 33$, power = 50%, $p < 0.05$). Only correlations that exceeded their sample-specific effect sizes were presented or discussed as statistically significant.

Results. Examination of demographic characteristics showed no statistically significant differences between left and right MTLE groups for chronologic age, education, gender distribution, and handedness (see table 1). Patients with left MTLE had higher (abnormal) left Cr/NAA hippocampal ratios, whereas significantly higher right Cr/NAA hippocampal ratios were found in the right MTLE patients. Left MTLE patients were younger at seizure onset and had a longer duration of epilepsy (see table 1). The two groups did not differ across measures of psychometric intelligence, BNT, LM immediate, VR immediate and VR % recall, and BFRT. However, left MTLE patients recalled significantly less across a 30-minute delay on the LM test compared to the right MTLE patients (see table 1).

Pearson correlations between the neuropsychological tests and the left and right Cr/NAA ratios are presented in table 3. Relationships were found between the left Cr/NAA hippocampal ratio and LM % recall ($p < 0.002$, figure 1) and the BNT ($p < 0.004$). The left Cr/NAA hippocampal ratio was negatively associated with both verbal memory measures with larger ratio values associated with poorer memory scores. The BFRT score was the only other neuropsychological measure to meet our a priori correlation value and was found negatively associated with the right Cr/NAA hippocampal ratio ($p < 0.02$, figure 2).

None of the predictor variables reached threshold values to enter into the stepwise multiple regression analyses for VR immediate, VR % recall, PASAT, VIQ, or PIQ (see table 2). However, age at seizure onset accounted for a significant amount of the explained variance predicting the LM immediate recall score. Younger seizure onset was re-

Table 2 Regression analyses predicting neuropsychological performance using creatine to N-acetylaspartate (Cr/NAA) hippocampal ratios, seizure onset, and seizure duration

DV/predictor	R ²	F ^a	p Value	β	t ^b	p Value
Verbal IQ		(No variable entered)				
Performance IQ		(No variable entered)				
LM immediate	0.22	8.4	0.007			
Seizure onset				0.47	2.9	0.007
LM % recall	0.28	12.0	0.002			
LH Cr/NAA ratio				-0.53	3.5	0.002
Boston Naming Test	0.18	9.1	0.004			
LH Cr/NAA ratio				-0.42	3.1	0.004
Facial recognition	0.12	5.8	0.02			
RH Cr/NAA ratio				-0.35	2.4	0.02
VR immediate		(No variable entered)				
VR % recall		(No variable entered)				
PASAT		(No variable entered)				

DV = dependent variable; LM = logical memory; VR = visual reproduction; PASAT = Paced Auditory Serial Additions Task.

lated to poorer immediate recall score. The left hippocampal Cr/NAA ratio accounted for a significant amount of the explained variance (%) for both the LM % recall score and the BNT score. On the measure of facial recognition, the right hippocampal Cr/NAA ratio explained a significant amount of variance (%) in performance. Larger right Cr/NAA hippocampal ratios were associated with poorer ability to identify unfamiliar faces.

Discussion. Previous research has shown the clinical utility of ¹H MRS imaging in efforts to lateralize epileptogenic temporal lobe tissue and to detect subtle neurochemical abnormalities not found with standard quantitative MRI techniques.^{12,13,15} However, only limited efforts have been directed at the examination of neurobehavioral correlates with these spectroscopic imaging techniques. The current study found significant associations between metabolic

markers of hippocampal neuronal integrity and selective measures of neuropsychological functioning in patients with mesial temporal lobe epilepsy. We found that the most striking relationship was between the left Cr/NAA hippocampal ratio and the ability to freely recall the content of the logical memory stories after a delay. No clinical variable or right Cr/NAA ratio was significantly associated with delayed prose recall. Age at seizure onset has been previously found associated with immediate verbal memory in the form of prose passages.^{28,29} These findings support the important contribution made by the left hippocampus to delayed prose recall and are consistent with previous studies using MRI volumetric measurements of hippocampal tissue and histopathologic cell count analysis.^{5,30,31} These results do

Table 3 Intercorrelations among the magnetic resonance spectroscopy creatine to N-acetylaspartate (Cr/NAA) ratios and neuropsychological measures

Characteristic	LH Cr/NAA ratio	RH Cr/NAA ratio
LM Immediate	-0.03	-0.02
LM % recall	-0.53 ^a	0.19
BNT	-0.43*	0.07
VR Immediate	-0.10	0.21
VR % recall	-0.11	0.21
BFRT	-0.05	-0.33†
Verbal IQ	-0.17	0.05
Performance IQ	-0.06	0.13

LM = Wechsler Memory Scale logical memory; BNT = Boston Naming Test; VR = Wechsler Memory Scale visual reproduction; BFRT = Benton Facial Recognition Test.

*p < 0.005; †p < 0.05.

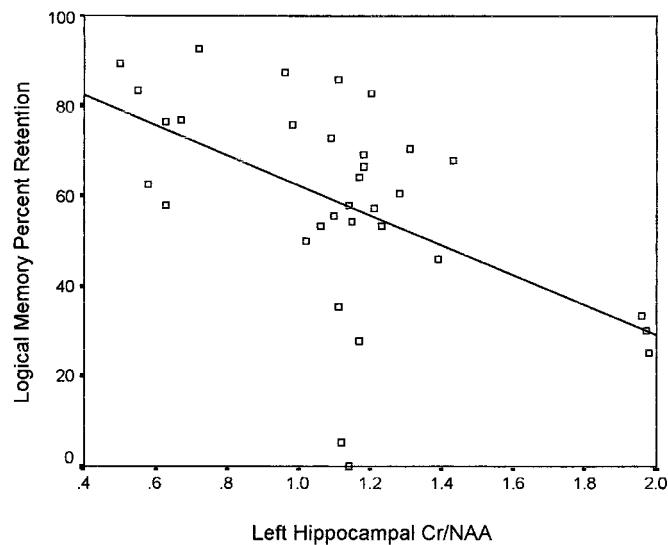


Figure 1. Scatterplot depicting the relationship between logical memory percent retention and the left hippocampal creatine to N-acetylaspartate ratio.

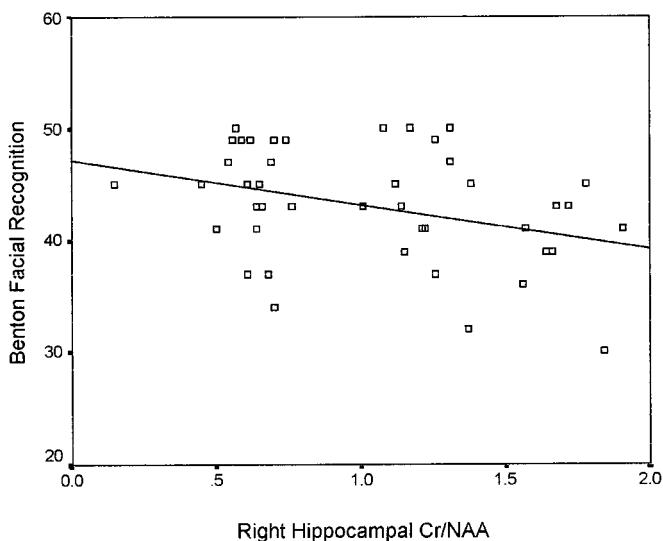


Figure 2. Scatterplot depicting the relationship between Benton Facial Recognition Test and the right hippocampal creatine to N-acetylaspartate ratio.

not preclude other temporal and extratemporal areas from playing an important role in prose recall as evidenced by significant associations being found between left lateral temporal lobe and left thalamus glucose metabolism (FDG-PET) and logical memory-delayed recall in a study of mesial temporal lobe epilepsy patients.³²

The current results indicate that the left hippocampus is a vital component in the process of visual confrontation naming. We found that metabolic functioning of the left hippocampus was strongly associated with visual confrontation naming performance as measured by the BNT. The strength of this association was nearly as strong as that found between the delayed story recall and left Cr/NAA hippocampal ratio and indicates that confrontation naming uses cognitive resources supported by the hippocampus. This was contrary to a previous study that used hippocampal cell count/density estimations in which no association between tasks of visual confrontation naming and the left hippocampus was found.³¹ However, our findings do confirm previous research, which has found that MTLE patients with isolated left hippocampal lesions show impaired confrontation naming performance and perform more poorly than do MTLE patients without sclerotic left hippocampal tissue.^{3,33,34} Confrontation naming has been previously shown to result in large areas of brain involvement during functional activation studies (i.e., PET), which incorporate a neural network involving bilateral visual cortex, inferotemporal and hippocampal areas, and inferior frontal gyrus (relatively exclusive left-sided activation).^{35,36} Compared to control subjects, reduced cerebral blood flow activation of left fusiform gyrus has been reported in temporal lobe epilepsy patients who are identified as experiencing visual naming impairments. Stimula-

tion of left basal temporal areas also impairs naming performance.^{37,38}

We found that neither right or left Cr/NAA ratio nor clinical variables accounted for a significant amount of explained variance on the Verbal or Performance IQ from the WAIS-R. This is consistent with previous reports on the null relationship between psychometric measurement of intelligence and mesial temporal structure.³⁰ This finding is also consistent with some reports indicating that patients experiencing dense bilateral hippocampal pathology are able to acquire some aspects of semantically complex information despite profound anterograde amnesia.¹¹ Most of our patients were able to complete high school despite their epilepsy and memory problems. Additionally, our measure of attention was unrelated to Cr/NAA hippocampal ratios. Generally, the PASAT scores were within normal range and are consistent with previous reports of normal to near-normal attentional performance in MTLE adult populations.³

We failed to find any association between VR immediate or VR % recall scores with either right or left Cr/NAA hippocampal ratios. This is consistent with recent data indicating a lack of relationship between the right hippocampus and certain aspects of visual memory. No relationship was found between the visual memory test and age of seizure onset or seizure duration. Several authors have pointed out that tasks such as VR are not specifically sensitive to right mesial temporal lobe dysfunction and that such measures are susceptible to both verbal and nonverbal encoding strategies (i.e., "dually encodable").⁶ However, some studies have reported positive associations between right hippocampus and nonverbal tasks that rely more on the processing of spatial information or less readily verbalizable visual stimuli.³⁹ The current study did not use a measure of spatial memory, which may have been found sensitive to the right Cr/NAA hippocampal ratio. Additionally, certain studies have reported that extrahippocampal structures (i.e., parahippocampal gyrus) may provide a critical link toward understanding the ability to encode spatial information for long-term storage.⁴⁰ Such extrahippocampal structures were not measured in the current study and await further examination.

Only the BFRT correlated significantly with the right hippocampal Cr/NAA ratio. This relationship was weaker than the left hippocampal Cr/NAA and verbal memory correlations but still reached significance when entered into our regression equation. Neither age, seizure onset, seizure duration, nor left hippocampal Cr/NAA ratio explained a significant amount of statistical variance. This was somewhat unexpected as previous literature on facial perception has identified extrahippocampal structures such as the fusiform gyrus within the inferotemporal area as playing a central role in identifying facial specific features.⁴¹ Several reports on patients with bilateral temporal-occipital lesions have found profound re-

sulting prosopagnosia.⁴² Functional neuroimaging studies have shown bilateral temporal-occipital activation involving the amygdala, inferior-middle-superior temporal gyri, as well as anterior mesial temporal activation during processing of facial stimuli.²⁶ Sergent et al.⁴¹ showed that in control subjects, bilateral activation of bitemporal areas occurred during facial processing, although primary activation was noted in the right parahippocampal and right anteromedial temporal lobe. Kapur et al.⁴³ point out that automatic aspects of facial processing (whether familiar or unfamiliar) activate the right parahippocampal gyrus, a finding we may have found if we had sampled this area. However, it is possible that the functional metabolic disturbance may derive from adjacent parahippocampal dysfunction since some voxel contamination is likely. Additionally, another possible explanation for the current findings is that the right hippocampal Cr/NAA may have been correlated with Cr/NAA of another temporal structure that lies well outside the right hippocampal area and that this other temporal lobe region is involved in visual recognition processing, while the right hippocampus is not. Future studies will need to expand the area of interest using ¹H MRSI to include those other areas involved in facial recognition processing to fully delineate the hippocampal contribution.

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Methodologic issues in assessing risk factors for epilepsy in an epidemiologic study in India

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Article abstract—Objectives: To test a field questionnaire for epilepsy risk factors in rural India. **Background:** Case-control studies are necessary to plan appropriate prevention and intervention strategies against epilepsy but difficult to mount in developing countries for various logistic and cultural reasons. Recent studies have proposed nutritional and infective risk factors not reported in Western studies. **Methods:** A structured questionnaire including known and putative risk factors was administered to parents of 61 children with epilepsy and 59 randomly selected population control subjects in rural West Bengal. ORs of effect were calculated using a multiple logistic regression model with 95% CIs. **Results:** Socioeconomic status, febrile convulsions, and reproductive history were easily assessable. Ages, dates, and some proxy infection markers were unreliable. Febrile convulsions were an independent risk factor (OR 6.45; 95% CI, 1.45 to 28.66). Associations with family history and infective symptoms were suggested. No association was demonstrated with socioeconomic status or reproductive factors. **Conclusions:** Known risk factors were confirmed, and novel factors were suggested. A retrospective questionnaire does not allow accurate study of infective exposures. Greater statistical power is needed to study weaker associations and interacting effects. **Key words:** Epilepsy—Childhood—Risk factors—Epidemiology—Infection.

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Epilepsy affects 5 to 10/1,000 population throughout the world, with 75% of cases arising in childhood.^{1,2} Incidence rates are higher in developing countries, with the highest prevalence from these regions.² Approximately 70% of epilepsies are of unknown cause in Western studies.³

Studies in Ecuador, Tanzania, Nigeria, and Pakistan have consistently found higher prevalences of epilepsy in rural compared with urban areas using the same methodologies,^{4–7} suggesting that infectious diseases may be an important etiologic factor in epilepsy in the developing world. Most notable are the Latin American population studies, which have highlighted the contribution of neurocysticercosis to late-onset epilepsy.^{8–10}

The association between clinical and subclinical infection and epilepsy is important to explore in de-

veloping countries. The case-control design is a powerful, quick, and simple method of examining and quantifying disease exposure associations.¹¹ Much of what is known about the causes of epilepsy and the separation of syndromes derives from large population case-control studies such as in Rochester, Minnesota³ and the National Collaborative Perinatal Project.¹² Studies of similar scale are necessary in developing countries to identify the distribution of risk factors, which are likely to vary from region to region.² Any such survey would rely on questionnaire-based methods as a primary source of exposure data. The case-control design has been applied infrequently in developing countries.¹³ Field studies in developing countries are beset by problems of logistics, resources, and variant public health priorities and cultural expectations among respondents. This

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may partly explain why there are so many descriptive studies, and so few analytic studies. One of the main difficulties facing the researcher in developing countries is the absence of routine data or medical records on which to base estimations of past exposure. Birth dates and ages may not be registered; literacy levels are sometimes low; and diagnoses may not be explained to patients when discharged from hospital or clinic, making historical data unreliable. Directly administered questionnaires may be unfamiliar to respondents in certain parts of the world, which can lead to problems in data collection and information bias. Undesirable effects on the community caused by surveys must also be considered.¹⁴ Thus, it would be informative to pilot the questionnaire method to assess potential methodologic difficulties.

The aim of this study, therefore, was to test a questionnaire to be used in a field epidemiologic study into the etiology of epilepsy in rural India. Previous case-control studies have demonstrated febrile convulsions, head injury, meningoencephalitis, and family history as risk factors. Interesting associations have also been reported with reproductive factors, low body mass index, and, possibly, lack of immunizations.^{13,15-17} The questionnaire attempted to measure infectious exposure and reproductive risk. These two exposures in particular were chosen because infectious diseases are more common in developing countries, and childbirth and pregnancy more hazardous than in the West. One objective was to assess how well proxy markers for infectious contact risk performed. These markers included overcrowding, immunization completeness, animal contact, household hygiene, and use of bed netting. The study also investigated association with febrile convulsions, family history of recurrent seizures, head injury, and socioeconomic status.

Methods. Design. A structured questionnaire was administered to the mothers of 61 children with epilepsy and 59 random control subjects 2 to 18 years of age. Data were analyzed as an unmatched case-control study.

Geography. West Bengal is a large state in eastern India composed of 17 districts. The study took place in 46 villages of Bishnupur Blocks I and II in district 24 Parganas South, a flat, rural district south of Calcutta with a population of 117,000 and an area of 213 km². Income is seasonal, mainly from farming, the per capita gross domestic product is \$380, the female literacy rate 36%, and the infant mortality rate is 65 per 1,000 live births. The crude population growth rate is 20 per 1,000 per year.¹⁸ Families are mainly nuclear, with a mean size of 6, but intergenerational ties are strong. Most (59%) of the population are Hindu; 40% are Muslim; and there is a 1% Christian minority. The study took place in the context of a primary-level epilepsy treatment program. This was provided by a maternal and child health clinic (Child in Need Institute) and a community based rehabilitation organization (SANCHAR-AROD).

Subjects. Children with untreated epilepsy were identified by two-stage screening methods from a population of

40,000 children between August 1995 and February 1996.¹⁴ Both key informant and house-to-house survey methods were used in the first stage, and a pediatric neurologist (the author) verified the diagnosis clinically, without EEG, using operational definitions for epilepsy and seizure type.^{19,20} Using this method, 132 children between the ages of 2 and 18 years who experienced epileptic seizures were identified; 104 had untreated epilepsy (more than two seizures in the previous year), and 94 of these entered the treatment program, which incorporated a clinical trial of phenobarbital and phenytoin for generalized tonic-clonic or partial seizures.²¹ Sixty-one of these children were selected from the clinic register if they attended 4 consecutive weeks in March 1996. Fifty-nine control subjects were selected from the population register by random sampling, stratified by village, using random number tables.

Measures and procedures. The questionnaire was divided into five sections: 1) demographics; 2) socioeconomic status; 3) reported risk factors; 4) reproductive factors; and 5) proxy infection markers.

Demographics: Age to the nearest year, sex, family size, and village were recorded.

Socioeconomic status: An index (range, 0 to 16) was constructed from maternal literacy based on ability to read, write, and tell time (none/basic, intermediate, or proficient), occupation of head of household in four standard categories (daily wage earner, business, skilled trade, or salaried), house tenure (owned or rented), ownership of land (none/unfarmed/farmed), type of house (brick or earth), roof material (tin or concrete), electricity supply and household possessions (list, including radio, bicycle, livestock). Similar methods of assigning socioeconomic status have been used in field studies in Bangladesh.^{22,23}

Reported risk factors: A history was taken for febrile convulsions, defined as in international epidemiologic guidelines¹⁹: first-degree family history of recurrent seizures; and severe head injury with prolonged (24 hours) unconsciousness as defined in epidemiologic studies in Rochester.^{16,17} Mild or moderate head injury was not studied because of the anticipated difficulty of misclassifying minor head injuries with brief or no unconsciousness.

Reproductive factors: A history was sought for previous maternal miscarriage, stillbirth or neonatal death, or maternal vaginal bleeding during early pregnancy with the index child.

Proxy infection markers: Potential risk of airborne, fecal-oral, or vector-borne infective exposure was measured using operationally defined proxy indicators: household crowding (>6 occupants per room); number of immunizations (checked against record card, maximum six: diphtheria + pertussis + tetanus + polio $\times 3$, preschool booster of diphtheria, tetanus, and pertussis [DTP], and polio, measles, and bacillus Calmette-Guerin [BCG]); frequent contact with domestic animals such as cattle, poultry, goats, cats, and dogs (yes/no); infective illness (fever, diarrhea, or coryza) in the 4 weeks before interview (yes/no); nightly bed netting use (yes/no); and presence of a sanitary toilet.

Table 1 Demographic characteristics

Characteristic	n (%)
No. of subjects	59
Sex	
Male (%)	30 (51)
Female (%)	29 (49)
Age, y	
Median (range)	8 (5–14)
25th–75th quartile	7–10
Number of rooms (%)	
1	47 (80)
2	12 (20)
>2	0
Inhabitants (%)	
2–4	24 (41)
5–6	23 (39)
7–12	12 (20)
Children in family (%)	
1–2	23 (39)
3–4	29 (49)
5–8	7 (12)

All data were collected by a specially trained, married, female interviewer blinded to the study hypothesis. The questionnaires were administered to mothers verbally to overcome problems of literacy. A woman interviewer was chosen because she would have a better chance of gaining access to households, particularly to mothers, and be able to ask sensitive questions about reproductive function. Her marital status was important to gain respect and sanction from male family members. Five field interviews were conducted per day, each taking approximately 30 minutes to complete. Data were collected for control subjects at home, and for cases, in the clinic.

Statistical analysis. The full data set was analyzed in an unmatched case-control design using univariate Mantel-Haenszel methods. The following risk factors were examined: febrile convulsions; first-degree family history of epilepsy; severe head injury with loss of consciousness for more than 24 hours; socioeconomic score; previous maternal stillbirth or neonatal death; maternal vaginal bleeding in index pregnancy; overcrowding (more than six people per room); fewer than three immunizations; frequent animal contact; infective illness in the previous 4 weeks; regular bed netting use; and logarithm of body mass index plus one. Significant risk factors were then entered into a multiple logistic regression model, using Stata 5.0 for Macintosh software.²⁴ ORs for effect, adjusted for age and other confounding variables, were reported with 95% CIs.

Results. The responses from control families are summarized in tables 1 and 2.

Demographics. Fifty-one percent of children were male. Their ages were obtained to the nearest year because very few had recorded birth dates. The median age

Table 2 Socioeconomic indicators

Indicator	n (%)
Maternal literacy	
None/basic (%)	30 (51)
Intermediate (%)	19 (32)
Proficient (%)	10 (17)
Occupation of head	
Daily wage (%)	40 (68)
Business (%)	8 (14)
Skilled trade (%)	2 (3)
Salaried (%)	9 (15)
House tenure	
Rented (%)	4 (7)
Owned (%)	55 (93)
Land	
None (%)	43 (73)
Unfarmed (%)	0
Farmed (%)	16 (27)
House type	
Earth (%)	43 (73)
Brick (%)	16 (27)
Roof	
Thatch or tile (%)	3 (5)
Cement or tin (%)	56 (95)
Toilet	
Field/semifield (%)	38 (64)
Sanitary (%)	21 (36)
Electricity supply	
Present (%)	15 (25)
Possessions	
Livestock (%)	43 (73)
Radio (%)	29 (49)
Tape recorder (%)	4 (7)
Bicycle (%)	21 (36)
Television (%)	5 (8)
Pond (%)	42 (71)

was 8 years with a range from 5 to 14 years. Almost half of families (49%) had three to four children.

Socioeconomic status. Half of mothers (51%) had no functional literacy, and most men (68%) were farmers or daily wage earners (table 2). Most (93%) owned their own house, usually made of earth (73%), with a cement or tin roof (95%), usually without any associated land (27%). Most had a pond (73%) and livestock (73%). Only 25% of households had an electricity supply. A radio (49%) and a bicycle (36%) were the most common household possessions. Few owned a television or a tape recorder.

Reported risk factors. A history of febrile convulsions was present in 5% of children (table 3). The precise age in months at the time of the convulsion was not usually recalled. No head injury was reported in this

Table 3 Risk factors and univariate associations with childhood epilepsy

Risk factor	Control subjects	Cases	OR (95% CI)
Socioeconomic score, mean (SD)	8.6 (3.9)	8.3 (4.4)	0.99 (0.91–1.08)
History of febrile convulsions (%)	3 (5)	11 (18)	4.11 (1.05–16.1)
First degree family history of epilepsy (%)	2 (3)	5 (8)	2.55 (0.47–13.89)
Head injury with unconsciousness >24 h (%)	0	1 (2)	∞
Previous maternal stillbirth or neonatal death (%)	16 (27)	12 (20)	0.67 (0.28–1.59)
Maternal bleeding in index pregnancy (%)	0	7 (12)	∞
Overcrowding, seven or more per room (%)	8 (14)	7 (12)	0.83 (0.28–2.46)
Frequent animal contact (%)	45 (76)	39 (64)	0.55 (0.25–1.24)
Regular bed net use	52 (88)	54 (89)	0.96 (0.31–2.95)
Immunizations, fewer than three (%)	7 (12)	19 (32)	3.44 (1.28–9.28)
Infective illness in previous 4 weeks (%)	35 (59)	50 (82)	3.12 (1.31–7.40)
Log body mass index geometric, mean (SD)	0.34 (0.14)	0.26 (0.23)	0.11 (0.01–0.92)

group. First-degree family history of epilepsy was reported in 3% and a first-degree family history of febrile convulsions in 8% of children. A specific maternal history of seizures was not separately recorded.

Reproductive factors. Neonatal and fetal deaths were common, occurring in 27% of mothers. Prenatal hemorrhage was not reported in this group.

Proxy infection markers. Fourteen percent of families had seven or more members per room; 12% of children had received fewer than three immunizations; 76% had frequent animal contact, usually with stray dogs and cats, or when taking cattle out to pasture; 59% had a history of an infective illness in the previous 4 weeks; 88% regularly used a bed net; and 36% had a sanitary toilet.

Sex distribution was equal among cases (48% boys) and control subjects (51% boys). Cases were older (median, 12 years; interquartile range, 3 to 16 years) compared with control subjects: there were no significant differences among items contributing to the socioeconomic score such as maternal literacy, head of the household's occupation, physical quality of the house, and the number of household possessions. Consequently, the univariate OR for socioeconomic status was very close to unity (0.99; 95% CI, 0.91 to 1.08).

Table 3 compares the frequency of risk factors in case and control groups. Febrile convulsions were reported in 18% of cases and 5% of control subjects, resulting in an OR of 4.11 (95% CI, 1.05 to 16.1). A first-degree family history of epilepsy was present in 8% of cases and 3% of control subjects (OR 2.55; 95% CI, 0.47 to 13.89). Severe head injury was reported in one case but no control subjects, so the OR could not be calculated. No other head injuries were reported. Maternal stillbirths and neonatal deaths were common, occurring in approximately one fourth of mothers of both cases (20%) and control subjects (27%). Vaginal bleeding in pregnancy was reported only in mothers of cases and not of control subjects.

There were no differences in overcrowding between case and control families (12% and 14%, respectively, having more than six occupants per room). Contact with animals was very common, in 64% of cases and 76% of control subjects. A high proportion of cases (89%) and control subjects (88%) slept under a bed net. Thirty-two percent of

children with epilepsy had three or fewer immunizations, compared with 12% of control subjects (OR 3.44; 95% CI, 1.28 to 9.28). Cases were also more likely (82% versus 59%) to have had infective symptoms in the 4 weeks before interview (OR 3.12; 95% CI, 1.31 to 7.40). Differences in measures of body mass index (OR 0.11; 95% CI, 0.01 to 0.92) disappeared after adjusting for age (OR 31.3; 95% CI, 0.8 to 1203).

There were significant associations of epilepsy with febrile convulsions, infective illness, and immunizations in univariate analysis. The association with family history of epilepsy was nonsignificant, probably because of the small sample size. These four risk factors were entered into a multiple logistic regression model, adjusting first for age and then all confounding variables (table 4). Head injury and prenatal hemorrhage could not be studied further in this way because of the absence of exposure in control subjects. In multivariate analysis, febrile convulsions were a strong independent risk factor (OR 6.45; 95% CI, 1.45 to 28.66). Family history of epilepsy was not significant (OR 1.96; 95% CI, 0.32 to 12.03). The effect of immunizations was partly confounded by age differences between cases and control subjects and lost significance in multivariate analysis (OR 1.98; 95% CI, 0.65 to 6.02). To test this further, only children younger than 12 years of age were compared, and no differences in immunization between cases and control subjects were found (88% versus 89%, respectively). Confounding by age was therefore a probable

Table 4 Summary age-adjusted and multivariate odds ratios of effect

Risk factor	Age-adjusted OR (95% CI)	Multivariate OR* (95% CI)
Febrile convulsions	7.05 (1.63–30.47)	6.45 (1.45–28.66)
Family history epilepsy	2.09 (0.37–11.91)	1.96 (0.32–12.03)
Immunizations, fewer than three	2.00 (0.70–5.75)	1.98 (0.65–6.02)
Infective illness in previous 4 weeks	3.13 (1.30–7.52)	3.06 (1.20–7.81)

* Adjusted for febrile convulsions, family history, immunizations, infective illness, and age.

explanation for the association with immunization. Infective illness retained a strong independent association (OR 3.06; 95% CI, 1.20 to 7.81).

Discussion. *Use of questionnaire.* The general description of the study sample is informative about the socioeconomic conditions that typify life in rural India. Households were quite large, crowded, and lacking basic amenities such as running water, electricity, and a sanitary latrine. However, a wide range of housing quality existed, some homes well built and equipped with all utilities. The head of the household was commonly a subsistence wage earner, and mothers had low literacy levels and suffered numerous adverse pregnancies. Socioeconomic indicators were directly characterizable and therefore more reliable than reported incomes, which may have been exaggerated or minimized by respondents.

A history of febrile convulsions, described by the specific Bengali term *tarka*, was obtained in 5% of control subjects. Operational definitions were used to reduce misclassification. Because the prevalence in control subjects was comparable with that in many other studies,^{6,13,17,25} it seems unlikely that episodes of malaria or febrile syncope were misclassified as febrile convulsions in this sample.

Family history of epilepsy may have been underestimated because of concealment, recall bias in older generations,²⁶ and other reasons. The importance of asking several informants for a family history of seizures to improve ascertainment has been emphasized.²⁷ Our previous study suggested that over 40% of epilepsy cases were missed even after thorough screening of the whole population.¹⁴ Maternal history of epilepsy is an important risk factor for epilepsy in offspring,²⁸ but was not separately recorded and would be very hard to elicit owing to concealment and recall difficulties.

Stillbirths and neonatal deaths were very common compared with Western populations. It was possible that early fetal losses would not be recalled, and therefore relatively memorable events had been chosen as items in this questionnaire, such as significant loss of blood in early pregnancy, fetal loss, stillbirth, or neonatal death. No prenatal hemorrhage was reported by control subjects in the first trimester. Obstetric events may have been underreported in the relatively open village environment, where people naturally gather around visitors with questionnaires. Women sometimes appeared reticent in answering direct questions from interviewers, especially in the presence of men. However, they were not inhibited in reporting stillbirths, suggesting that significant underreporting of obstetric events probably did not occur. Alternatively, the absence of reported prenatal hemorrhage could have been confounded by low maternal age or small number of pregnancies, neither of which had been recorded.

The presence of a sanitary toilet did not necessarily correlate with its use or with associated hand-washing and disinfective practices. Second, the toilet

was sometimes donated as part of a child sponsorship package of medical and educational assistance, and therefore acted as a significant confounding variable, and so was not analyzed further. This insight might not have been apparent without local knowledge.

In general, recall of age, time, and medical data was often vague and routine records were sparse. For example, precise maternal age would be difficult to record because of recall difficulties and an observed number preference for multiples of five. Recall of immunizations after 13 years was understandably difficult. The immunization record card was not always available and informants may have had a number preference for six (the maximum number of immunizations), especially if prompted. Measles and pertussis vaccines could not be distinguished, of potential interest because of associated encephalopathies. Medical records and routine health statistics were usually absent or incomplete.

The questionnaire used here was reliable for assessing unambiguous, unstigmatizing conditions such as demographics, socioeconomic status, and febrile convulsions. It also seemed to be satisfactory for measuring reproductive factors used here. The questionnaire method was deficient for recall items such as age, specific immunizations, past medical history, and family history. The intrusiveness of asking about reproductive and family histories underlines the need for sensitivity on the part of research teams, as well as the ethical dilemma involved in causing possible disturbance among families (especially control subjects) where no service or material gain is offered.²⁹ In this case, the study was part of a treatment program and disturbance was minimized to a degree, but this issue needs to be carefully considered in other settings.

The use of proxy markers for infection is fraught with difficulty and underlines the need for familiarity with the context in which the study is conducted and exquisite adaptation of data collection methods. Accurate study of infective exposures in this setting could not be achieved by retrospective questionnaire and requires either prospective data collection, or more proximate biomarkers such as acute-phase reactants, seroconversion, PCR techniques, and immunologic profiles.

Assessing risk factors. Febrile convulsions, described by the specific Bengali term *tarka*, were common among cases (18%). This was similar to findings in Tanzania (13%) and Rochester, Minnesota (18% to 19%), but lower than in Nigeria (29%).^{6,13,17} The effect size (OR 6.45; 95% CI, 1.45 to 28.66) was lower than in studies in Nigeria (OR 11.0) and Rochester for generalized and partial epilepsies (OR, 10.5 to 15.3), although CIs were wide. Simple and complex febrile convulsions could not be studied separately owing to sample size limitations. The association between febrile convulsions and epilepsy is well known,^{13,16,17} and may signify a genetic susceptibility or shared acquired pathologic process.

Cases were almost three times less likely to have been completely immunized. This was probably because control subjects were younger and would have had more chance of being immunized, because immunization programs in this part of India became widely available in the last decade. Birth order was not recorded in this study but provides an alternative explanation for the age differences. Previous reported association with immunization status may have been confounded by age or socioeconomic status.¹³

Particularly interesting was the association of recent infective illness with epilepsy. This phenomenon has also recently been reported from south India.³⁰ It might be explained by a neurologic disorder or its sequelae predisposing to chronic illness, some prior immunologic vulnerability that led to both infection and epilepsy, overreporting because of parental concern, or a chance finding. This finding merits further prospective research.

Body mass index, a measure of current malnutrition, was not associated with epilepsy. A previous study may have shown a false association because of a statistical error, failing to use logarithmic transformation for tests of significance and regression analysis.³⁰ Several studies have shown either no or univariate association with socioeconomic status.³¹⁻³⁵ The current study had a 60% power to detect a real effect and did not demonstrate an association, in keeping with the only other multivariate analysis, which used a similar measure.³⁰ Particular socioeconomic components may, however, be risk factors for epilepsy in other contexts or epileptic syndromes.

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