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ALTERED SUBICULAR MAP2 IMMUNOREACTIVITY IN SCHIZOPHRENIA

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A b s t r a c t: We wished to test independently a previously reported loss of subicular microtubule-associated protein 2 (MAP2) in the brains of deceased individuals who had suffered from schizophrenia, and to determine whether there were any clinical characteristics attached to such a loss. Immunohistochemistry for MAP2 was examined in the hippocampal region from 94 psychiatric patients: 64 with a primary diagnosis of schizophrenia or schizoaffective disorder, 12 with a primary diagnosis of major depressive or bipolar disorders, and 18 with a primary diagnosis of dementia; and from 17 individuals without psychiatric disease**.** Lifelong symptomatology was evaluated with the modified Diagnostic Evaluation After Death. Subicular MAP2 immunoreactivity was prominently depressed in 20% of schizophrenia cases, 8% of mood disorder cases, 22% of dementia cases, and in no nonpsychiatric cases. Among dementia cases, those with loss of subicular MAP2 immunoreactivity displayed more subicular gliosis, while

among the schizophrenia cases, there was no such association. Among schizophrenia subjects, loss of subicular MAP2 immunoreactivity was associated with fewer positive and negative symptoms over the course of the illness. Subicular MAP2 immunoreactivity is markedly diminished in a significant proportion of individuals chronically institutionalized for schizophrenia, and this does not represent a generalized destruction of subicular neurons. In contrast, among individuals institutionalized for dementia, loss of subicular MAP2 immunoreactivity is accompanied by gliosis. The loss of MAP2 immunoreactivity is associated with fewer clinical symptoms, suggesting that it may represent an adaptive response to schizophrenia. The chemical or structural abnormalities underlying decreased MAP2 immunoreactivity in schizophrenia remain to be determined.

Key words: dendrite, schizophrenia, subiculum, MAP2. Neuroscience classification codes: Disorders of the nervous system, Neuropsychiatric Disorders.

Introduction

In 1991, Arnold *et al.* [1] reported a dramatic loss of immunoreactivity for microtubule-associated protein 2 (MAP2) in the subiculum of 5 out of 6 autopsy brains from individuals with schizophrenia. This was also found in 1 out of 6 specimens from individuals with dementia, but in none of 6 normal control subjects. In 1997, we published preliminary findings of loss of subicular MAP2 immunoreactivity in 10 out of 36 schizophrenia cases (28%) [2]. There are reports for decreased MAP 2 immunoreactivity in other brain areas [3], and reports from one laboratory that certain epitopes of MAP2 may display increased immunoreactivity in schizophrenia [4, 5]. The disparate results among laboratories could be due to technical variables, such as tissue fixation and choice of antibodies. Alternatively, the differences could be the result of different populations of subjects, with different distributions of normal and reduced immunoreactivity.

There are other lines of evidence for dendritic abnormalities in schizophrenia. Golgi preparations demonstrate diminished arbors of the apical dendrites of subicular pyramidal cells, suggesting that diminished or altered MAP2 in these dendrites could be a cause or an effect of their altered morphology [6]. Similarly, Kalus *et al*. [7] demonstrated diminished arborization of the basilar dendrites of prefrontal pyramidal cells in schizophrenia. Several studies have found a loss of dendritic spines [6, 8, 9]. Decreased gray matter volume with preserved neuronal number has been reported in prefrontal cortex (BA 9, BA 10, BA 46), in the cortex as a whole, and in the primary visual cortex [10] "reviewed in". It appears that loss of neuropil, and particularly of dendrites, may be a widespread phenomenon in schizophrenia.

MAP2, normally found in dendrites and neuronal cell bodies, participates in the elongation of dendrites by stabilizing microtubules [11, 12]. Abnormalities of dendritic contacts in the subiculum, the major source of hippocampal output to neocortex, could have profound effects on behavior and cognition. In the current report, we examine the relationship between subicular MAP2 immunoreactivity and clinical course, as determined from detailed review of decades of clinical notes. We also explore how the methods and results of our own studies may be reconciled with other studies reporting greater and lesser alterations in MAP2 immunoreactivity.

Materials and methods

Subjects

The sample consisted of 64 schizophrenia or schizoaffective subjects, 12 mood disorder subjects (7 major depression, 2 with psychotic features, and 5 bipolar disorder, 3 with psychotic features), 18 subjects with a primary diagnosis of dementia, and 17 nonpsychiatric controls (Table 1). The psychiatric subjects had all been inpatients in New York State Office of Mental Health institutions and were autopsied between 1989 and 1994, either routinely or as part of a prospective study of elderly schizophrenics [13]. The selection criterion for these cases was a research diagnosis (see below) of schizophrenia, schizoaffective disorder, mood disorder, or dementia, and the absence of infarcts in the hippocampal formation. Nonpsychiatric controls were obtained from routine autopsies performed at Columbia-Presbyterian Medical Center in 1991 and 1992. Consent was obtained for all autopsies.

Table 1 – Табела 1

Demographic characteristics of sample
Демографски карактеристики на примерокот

Group	N	% female	age: mean (SD)	postmortem interval: hours, mean (SD)	length of fixation: days, mean (SD)
nonpsychiatric	17	59	59.9 (14.4)	25 (19)	~14
schizophrenia	64	36	75.2 (11.9)	56 (68)	40 (40)
mood disorder	12	67	77.0 (5.1)	76 (84)	77 (75)
dementia	18	67	76.3 (9.6)	46 (48)	61 (60)

Clinical diagnoses

Research diagnoses for 38 psychiatric subjects (29 schizophrenia, 3 mood disorder, 6 dementia) were established prospectively by review of records and by interviews with the subjects and informants, as described previously [13]. Review of hospital records was performed on all other cases, including the nonpsychiatric subjects, by a team of psychiatrists and psychologists who had no knowledge of the autopsy results. Diagnostic evaluations were conducted using the modified Diagnostic Evaluation After Death (mDEAD), a chart review protocol that has shown high inter-rater reliability [14]. All cases were rated by at least two reviewers. The reviews were discussed by the entire clinical diagnostic team, at which point any disagreements between reviewers were resolved, and consensus diagnoses were determined. Routine autopsy cases were classified as nonpsychiatric only if there was no psychiatric diagnosis, or if the only psychiatric diagnosis was an adjustment disorder.

In the mDEAD, lists of positive and negative symptoms (Table 1) are rated as present or absent during 6 age periods: <26, 26–45, 46–55, 56–65, 66–75; and >75 years. All subjects were evaluated over the entire course of the illness, from onset until death. For all schizophrenia subjects evaluated with the mDEAD, we tabulated the number of positive and negative symptoms present in each epoch.

Diagnostic Neuropathology

Detailed neuropathological data were available for all cases. These were based upon both a complete diagnostic neuropathological review, and assessment of neuritic plaque and neurofibrillary tangle counts performed blind to all clinical and neuropathological information [15, 17].

For the neuropathological diagnosis of Alzheimer's disease, Khachaturian [18] criteria were applied to counts of neuritic senile plaques in the neocortex.

Cases were not used if there was an infarct in the subiculum. No other neuropathologic exclusion criteria were applied.

Immunohistochemistry

To minimize the time that the tissue had been in formalin, we stained sections (5 μ thick) from archived paraffin blocks of the hippocampus (at the level of the lateral geniculate body) that were taken at the time of initial neuropathological examination. The intervals between autopsy and paraffin embedding are summarized in Table 1.

The anti-MAP2 monoclonal antibody (Amersham Life Sciences, Arlington Heights, IL) recognizes a phosphorylation-independent epitope of

high molecular weight MAP2 (MAP2a and MAP2b) (Richard B. Vallee, personal communication). Staining of paraffin sections was enhanced by microwave antigen retrieval in citrate buffer [19]. Detection was by the avidin-biotinylated peroxidase method with the Vectastain ABC kit (Vector Laboratories, Burlingame, CA) and diaminobenzidine.

Cases from different groups were stained concurrently for MAP2, in a total of 3 staining runs, with sections from a few blocks repeated in different runs. After evaluation of all of these slides, an additional MAP2 staining run was performed on those cases for which appropriate sections from the original paraffin block were still available. Ninety-seven such cases were stained, and the distribution of immunoreactivity was identical to that in the original sections.

Immunohistochemistry for GFAP was carried out identically, in a single staining run, with a monoclonal antibody from Dako (Carpinteria, CA).

Evaluation of MAP2 immunoreactivity

All of the slides were coded before examination, and all evaluations were blind to clinical information and neuropathological findings. Before examining the MAP-2 stained sections, the borders of the subiculum were determined on an adjacent section stained with cresyl violet. The subiculum was distinguished from CA1 and the presubiculum by the presence of distinct and continuous internal and external pyramidal cell layers [20]; no attempt was made to define the prosubiculum (Figure 1). The subicular borders were drawn with a marking pen on the coverslip of the cresyl violet-stained section, which was then aligned with the immunostained section on a light box, and the boundary markings were copied onto the coverslip of the immunostained section, which was then examined under the microscope. Two observers (GR and AJD) independently examined all of the immunostained cases under the microscope and rated them for the presence or absence of severely decreased staining of the subiculum. Since overall staining intensity varied somewhat from case to case, the endfolium (CA4) was used as an internal control. In the few cases where disagreements were present, both raters reviewed the slides together and arrived at a consensus.

Evaluation of Immunoreactivity for GFAP

To explore whether a loss of MAP2 immunohistochemistry might be related to conventional features of tissue damage, we performed immunohistochemical staining for GFAP on 28 cases selected by clinical diagnosis and MAP2 results: 6 schizophrenia and 4 dementia cases with low subicular MAP2 immunoreactivity, and 11 schizophrenia and 7 dementia cases without low subicular MAP2 immunoreactivity. After confirming by microscopy that macros-

copically visible staining corresponded to immunoreactive cells and processes, without background staining, we placed all of the stained slides on a light box, and with the aid of a hand lens, ordered them by intensity of subicular GFAP immunoreactivity, blind to diagnosis and MAP2 results.

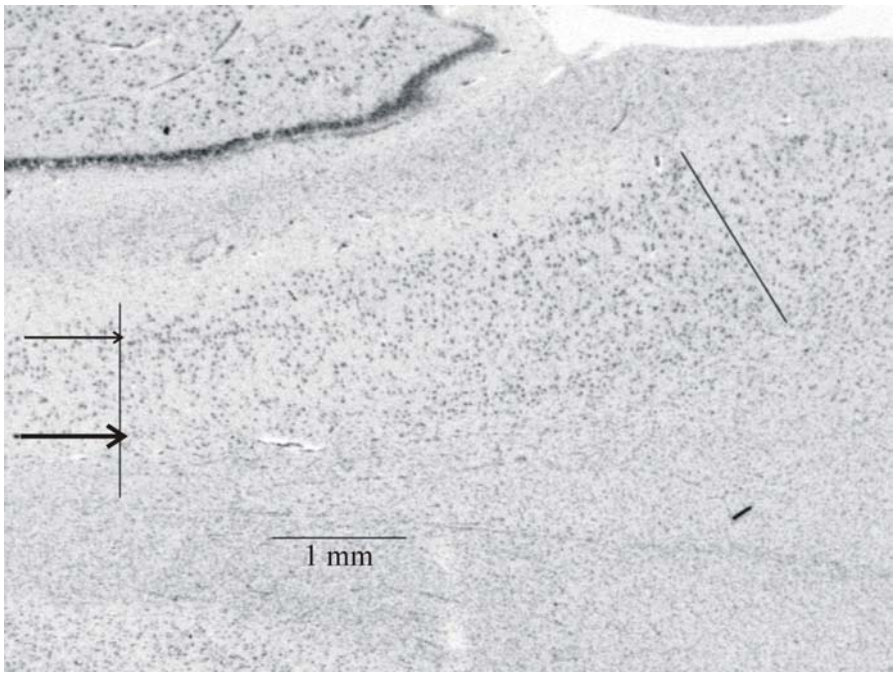

Figure 1 – *Boundaries of subiculum (vertical and diagonal lines). The subiculum was defined by the presence of a distinct and continuous internal pyramidal cell layer (thick arrow) and external pyramidal cell layer (thin arrow). (Also note proximity of CA4, upper left corner, surrounded by darkly stained dentate fascia.) Calibration bar = 1 mm*

Слика 1 – *Граници на субикулумот*

Data analysis

The main dependent variable was the qualitative rating of low subicular MAP2 immunoreactivity. Associations with clinical diagnoses, symptoms, treatments, and neuropathological findings were examined by χ^2 or 2-tailed Fisher's exact test (indicated below by p values with no test statistic). The hypothesis that low subicular MAP2 immunoreactivity was associated with higher GFAP ranks was tested by one-tailed Mann-Whitney U-test.

Results

MAP2 Immunohistochemistry

Immunohistochemistry for MAP2 resulted in intense labeling of neuronal cell bodies and dendrites in hippocampal field CA4 and in presubiculum and parasubiculum. In most cases, staining of the subiculum was comparable. On the other hand, in some cases, the subiculum contained very few stained structures or none. Staining was lost from both neuronal cell bodies and processes (Figure 2). Cases were classified as having low subicular MAP2 immunoreactivity when at least half of the mediolateral extent of the subiculum in the section was involved, while neuron and processes were clearly stained in CA4. In most cases so classified, the entire mediolateral extent of the subiculum was affected.

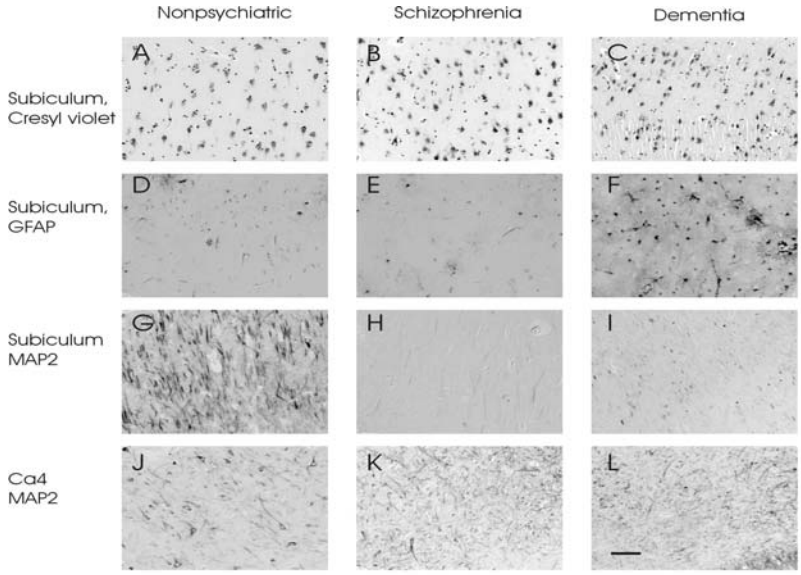

Figure 2 – *Nissl stain (a-c), immunohistochemistry for GFAP (d-f), and immunohistochemistry for MAP-2 (g-i) in central portion of subiculum, and immunohistochemistry for MAP-2 in CA4 (j-l). Nonpsychiatric case (a, d, g, j), schizophrenia case (b, e, h, k), and dementia case with neuropathologically-confirmed Alzheimer's disease (c, f, i, l). Calibration bar = 100 microns. Immunohistochemical stains (d-l) photographed under Nomarski optics*

Слика 2 – *Nissl боење (a-c), имунохистохемија за GFAP (d-f), имунохистохемија за MAP2 (g-i)*

Low subicular MAP2 immunoreactivity was present in none of the nonpsychiatric cases, 20% of the schizophrenia cases, 8% of the mood disorder cases, and 22% of the dementia cases ($\chi^2 = 5.1$, df 3, $p = 0.16$). In contrast to the subiculum, weak or absent immunoreactivity was a common finding in hippocampal field CA1. This was the case in 17% of the dementia cases and 40–42% of each of the other three groups. Low immunoreactivity in CA2 or CA3 was present in only two cases: one schizophrenia case with low subicular MAP2 immunoreactivity, and one mood disorder case with preserved subicular MAP2 immunoreactivity.

Since the nonpsychiatric subjects were younger and had shorter postmortem intervals (PMI) than the psychiatric subjects, we examined a subset of schizophrenia and control subjects aged < 70, with PMI ≤ 60 hours. Within this subset, mean (SD) age was 60.1 (10.6) years, and PMI was 13.1 (10.9) hours, both similar to the control group. The frequency of low subicular MAP2 (3 out of 14 cases, 21%) was as great as in the entire schizophrenia group (13 out of 64 cases, 20%).

MAP2 and GFAP

Subicular GFAP immunoreactivity was similar in schizophrenia cases with and without low subicular MAP2 immunoreactivity (Mann-Whitney $U = 29$, $W = 49$, $p = 0.48$). In the dementia group, however, there was more GFAP immunoreactivity in the cases with low subicular MAP2 immunoreactivity ($U = 2.0$, $W = 17.0$, $p = 0.07$) (Figures 2, 3).

MAP2 and neuropathological diagnoses

Although neuropathological findings were present in almost all of the psychiatric cases, there was no clear relationship between any neuropathological diagnosis, including both AD and acute infarction, and low subicular MAP2 immunoreactivity. Five schizophrenia cases with low subicular MAP2 were entirely free of neuropathological findings, and no abnormality of subicular MAP2 immunoreactivity was seen in five nonpsychiatric cases with infarction in other regions or acute ischemia, nor in one with demyelinating lesions. No schizophrenia case with low subicular MAP2 immunoreactivity met neuropathological criteria [18] for Alzheimer's disease. Average neocortical neuritic plaque counts per medium-powered field were lower in the schizophrenia cases with low subicular MAP2 immunoreactivity (0.07 (0.17)) than in those without (1.8 (3.7); $t = 3.26$, $df = 50.8$, $p = 0.002$).

Among the dementia cases with low subicular MAP2 immunoreactivity, one had a neuropathological diagnosis of Alzheimer's disease alone, one had Alzheimer's disease and infarcts, one had infarcts with senile degeneration that did not meet criteria for Alzheimer's disease, and one had

CNS Lyme disease [21]. The neuropathological diagnoses in the remaining dementia cases were: Alzheimer's disease alone (four cases), Alzheimer's disease plus infarcts (three cases), and one case each of senile degeneration not meeting criteria for Alzheimer's disease, senile degeneration plus infarcts, infarcts alone, Fahr's disease plus infarcts, encephalitis lethargica plus senile degeneration, and unclassified degenerative disease of central nervous system.

In two cases, no neuropathological lesions were identified.

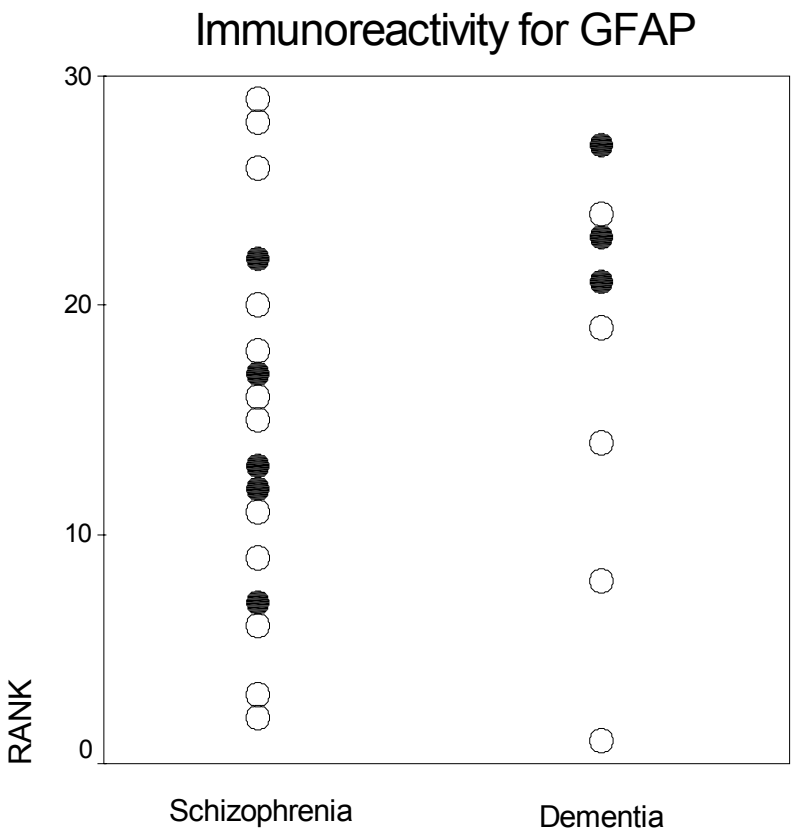

Figure 3 – *Ranked GFAP immunoreactivity for schizophrenia and dementia cases. Greater values indicate more GFAP immunoreactivity. Filled circles indicate cases with low subicular MAP2*

Слика 3 – *Рангирана GFAP имунореактивност за шизофрениите и деменциите*

MAP2 and clinical features

We found no relationship between MAP2 immunoreactivity and duration of agonal state. One schizophrenia case (with low subicular MAP2 immunoreactivity) died suddenly from trauma, and the others died of natural causes, either suddenly or in the course of an illness. Among 35 schizophrenia cases on which sufficiently detailed information was available, sudden deaths occurred with approximately equal frequency among those with (55%) and without (38%) low subicular MAP2 immunoreactivity (p=0.47). All of the nonpsychiatric cases died of natural causes, 4 suddenly and 13 following an illness.

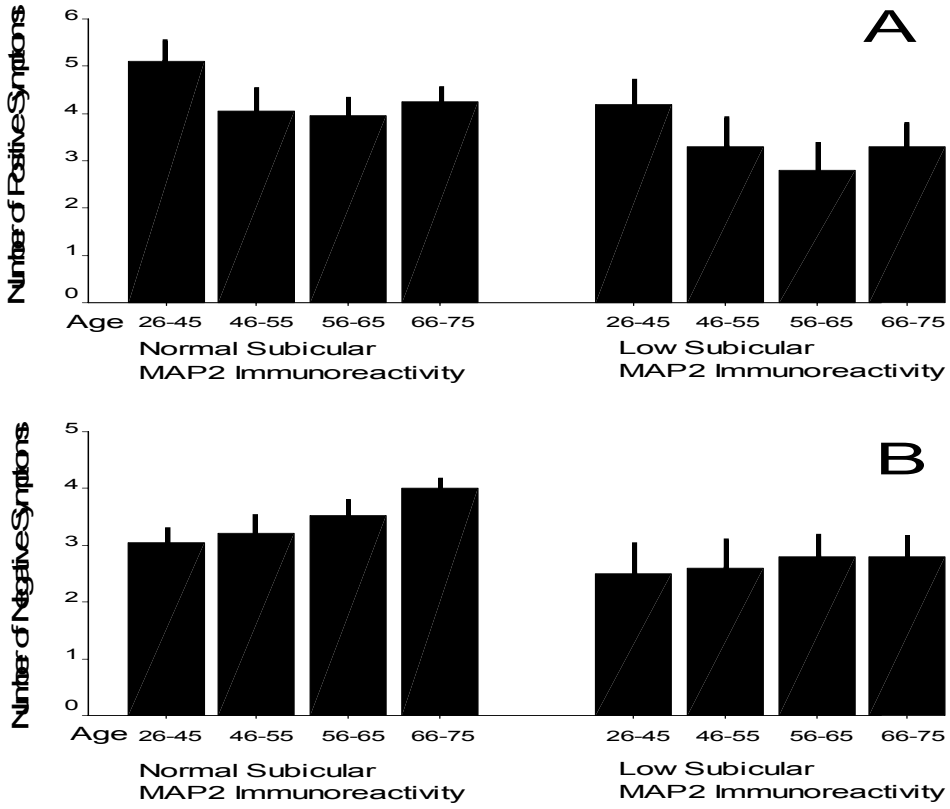

Figure 4 – *Average number of symptoms at each age for schizophrenia cases with normal or low subicular MAP2 immunoreactivity. Longitudinal study of 24 subjects with normal subicular MAP2 immunoreactivity, with 22 surviving to age 56–65, and 19 to age 66 or later; and 10 subjects with low subicular MAP2 immunoreactivity, all surviving to age 66 or later. Error bars = standard error of mean.*

Слика 4 – *Корелација на вредностите на MAP2 со симптомите кај пациентите со шизофренија*

Detailed lifetime clinical information was available from chart reviews of 35 of the schizophrenia subjects. All had been exposed to neuroleptic drugs. There was no apparent relationship between subicular MAP2 immunoreactivity and seizures, head injury, tardive dyskinesia, other movement disorder, cognitive impairment, psychiatric family history, or duration of illness. There was also no relationship between subicular MAP2 immunoreactivity and expo-sure to neuroleptics within one year of death or more than 10 years' lifetime exposure to neuroleptics.

Among 34 schizophrenia subjects who died at age 45 or later, and for whom the mDEAD was completed, subicular MAP2 immunoreactivity was associated with fewer symptoms over the course of the illness and at its conclusion. Adjusted for age at death, age at onset, and sex, (factors previously observed to be significantly associated with numbers of positive or negative symptoms; [22], the mean number of negative symptoms at the end of life was 54% lower for subjects with low subicular MAP2 ($p = .002$); positive symptoms were 33% lower, which was not statistically significant ($p = .13$).

Low subicular MAP2 immunoreactivity was seen more frequently in the lifetime *absence* of several of the 38 specific signs or symptoms of schizophrenia cataloged in the mDEAD. Without correcting for multiple comparisons, a significant relationships was found for only one clinical feature: low subicular MAP2 immunoreactivity was present in 21% and 71% of cases with ($n = 28$) and without ($n = 7$) loose associations (at any time in the illness), respectively ($p = 0.02$). Low subicular MAP2 immunoreactivity was also present in 22% and 63% of cases with ($n = 27$) and without ($n = 8$) poverty of speech or thought ($p = 0.08$), in 9% and 42% of cases with ($n = 11$) and without ($n = 24$) grandiose delusions ($p = 0.11$), and in 18% and 44% of cases with ($n = 17$) and without ($n = 18$) silly behavior ($p = 0.15$). No other relationship with a single symptom even approached statistical significance.

Sidedness and length of fixation

To minimize the period of fixation, this study used paraffin sections taken at the time of original neuropathological examination. Sections tended to be taken sooner after the autopsy for the nonpsychiatric brains than for the psychiatric brains (Table 1). To test whether fixation might be responsible for the localized subicular loss of MAP2 immunoreactivity, we stained an additional series of 14 general hospital cases that had been autopsied 1–5 months earlier. For these cases, in contrast to the 111 cases discussed above, clinical histories were obtained only from the autopsy reports. Cases with known neurological or psychiatric diseases were excluded. Fixation time for these specimens was 100 [23] days, with a range of 32–152 days. New sections of the hippocampal formation at the level of the lateral geniculate body were taken from the formalin-fixed tissue and embedded in paraffin, and sections of these were

then stained in parallel with sections from the original paraffin blocks, taken approximately 2 weeks after autopsy. The new sections were taken bilaterally, except in one case where only one hemisphere was available. The original sections had been taken bilaterally in 3 cases, from the left side in 3 cases, from the right side in 7 cases, and from an undetermined side in 1 case. There was no loss of subicular MAP2 immunoreactivity in either the original or the new sections, regardless of side.

Among the schizophrenia cases, 27 were known to have been sampled on the left side, and 33 on the right side. Low subicular MAP2 immunoreactivity was found in 8 (29.6%) of the left-sided sections and 3 (9.1%) of the right-sided sections. The side sampled had not been recorded for the original group of nonpsychiatric cases, but as already noted, the second group of nonpsychiatric cases showed no loss of subicular MAP2 immunoreactivity on either side, regardless of duration of fixation.

Discussion

Comparison with previous results

Like Arnold *et al*. [1], we found a prominent, localized loss of subicular MAP2 immunoreactivity in some cases of schizophrenia, but in no normal controls. While the patients in both studies were similar in terms of age, sex, treatment, and neuropathological findings, low subicular MAP2 immunoreactivity was much less frequent in our series. The studies employed different primary antibodies and different immunohistochemical techniques, which might account for the different outcomes.

Since there was no localized loss of MAP2 immunoreactivity in our first series of 17 confirmed nonpsychiatric cases, nor in our second series of 14 presumed nonpsychiatric cases, nor in the 6 nonpsychiatric cases reported by Arnold *et al*., it is very unlikely that our observation in schizophrenia represents normal variation. Further, since low subicular MAP2 immunoreactivity in schizophrenia was unrelated to gliosis, it is unlikely that this is a nonspecific sign of neuronal injury, as it may be in dementia cases. Rather, our data suggest that low subicular MAP2 immunoreactivity in schizophrenia is related to a more benign course of the disease. This suggests that low subicular MAP2 immunoreactivity may be associated with a successful adaptive response to schizophrenia, one that does not occur in the absence of disease. Alternatively, low subicular MAP2 immunoreactivity could be associated with a distinct etiology that leads to an attenuated clinical presentation.

Our knowledge of the relationship of specific MAP2 epitopes to dendritic remodeling and synaptogenesis is inadequate to predict a relationship between low subicular MAP2 immunoreactivity and synaptic plasticity in the

subiculum or elsewhere. The presence of lower neocortical neuritic plaque counts in the schizophrenia brains with low subicular MAP2 immunoreactivity suggests that this immunohistochemical finding may be related to enhanced formation or stability of neocortical synapses.

Cotter *et al*. [4] described small numbers of neurons immunoreactive for phosphorylated MAP2, without significant differences between schizophrenia and control subjects in the subiculum or any of the hippocampal subfields. While not commenting on the number of neurons immunoreactive for nonphosphorylated MAP2, they note that the cytoplasmic staining of immunoreactive subicular neurons was moderately more intense (~ 20% greater optical density) in 7 schizophrenia cases sampled on the left than in 5 control cases sampled on the left. This finding is not necessarily inconsistent with our results. Among 7 schizophrenia cases, we would expect only 1 or 2 to show a loss of MAP2 immunoreactivity, and even these might contain a rare neuron with intense immunoreactivity. However, in a subsequent study, based largely on the same samples, Cotter *et al*. [5] found subicular dendritic arbors in schizophrenia that were approximately 50% more extensive than controls when stained for nonphosphorylated MAP2, and approximately 20% more extensive when stained for total MAP2. This result is clearly inconsistent with ours and with those of Arnold *et al*. [1], since in our affected cases and those reported by Arnold *et al*., the loss of subicular MAP2 immunoreactivity was virtually complete. One possible explanation is that we and Arnold *et al*. used antibodies specific for high molecular weight MAP2, while Cotter *et al*. used antibodies that also detected the low molecular weight form, MAP2c. MAP2c is mainly expressed early in development [12], but persists in certain developmental abnormalities [24]. A more likely explanation for the difference between our results and those of Cotter *et al*. is that, rather than an increase or a decrease in MAP2, schizophrenia is associated with a physical alteration of the protein (such as its binding to microtubules; see below) that differentially affects the immunoreactivity of different epitopes.

Limitations of immunohistochemistry

The major limitation of all of the immunohistochemical studies so far is that the antemortem neurochemical basis for the observed changes in MAP2 immunoreactivity is not known. MAP2 immunoreactivity changes very rapidly after death, from a predominantly dendritic pattern seen only in biopsies or perfusion-fixed animals, to a somatodendritic pattern, which thereafter remains fairly stable [25, 29]. Immunoreactivity depends also on fixation and microwave treatment [26]. Finally, even without effects of postmortem delay or fixation, immunoreactivity can be affected by physical or chemical variations in an

antigen that affect the accessibility of individual epitopes. In some animal studies, immunoreactivity does appear to correspond, at least roughly, to amounts of MAP2 protein as measured by western blots [30, 31], but this has not yet been demonstrated for human post mortem material.

In this regard, it is also problematic that immunohistochemistry, while an excellent technique for localizing antigens, does not provide quantitation. As yet, quantitative analysis of images of immunohistochemical stains is of unproved validity. We do not know to what extent MAP2 must be reduced or antigenically altered to produce a complete loss of distinct staining. It is quite possible that even cases with a moderate loss of MAP2 produce normal-appearing immunoreactivity.

Because of these limitations and the presence of case-to-case variation in overall staining intensity, we used the staining of CA4 as an internal control to which we compared the subiculum. The results are similar if neocortex is used as the internal control; nonetheless, we cannot rule out the possibility that in some cases we interpreted an increase in CA4 immunoreactivity as a decrease in subicular immunoreactivity.

Somatic treatments

The psychiatric subjects in our study had received neuroleptic drugs, chronic institutionalization, and other treatments, while the nonpsychiatric subjects had not. While many subjects treated with neuroleptics did not show loss of MAP2 immunoreactivity, we cannot rule out the possibility that neuroleptic drugs affect MAP2 in some subjects. In the rhesus monkey, a year of haloperidol treatment resulted in statistically insignificant *increases* in MAP2 in most brain regions [32], but since the drug also produced a statistically significant increase in serine phosphorylation of MAP2, it is difficult to predict the net effect on immunoreactivity (see below). In the rat, two weeks of desipramine administration produced a similar increase in serine phosphorylation of MAP2. Such effects could contribute to the clinical actions of these drugs.

Possible significance of loss of subicular MAP2 immunoreactivity

Loss of MAP2 immunoreactivity is an early sign of irreversible injury leading to cell death from ischemia or hypoxia [33, 40], trauma [41, 45], excitotoxic agents [46, 47], and experimental seizure [48]. However, this is not a plausible explanation for the loss of immunoreactivity in schizophrenia, since these processes would eventually lead to severe neuronal loss and gliosis in the subiculum. We did not observe this, and it has not been reported by others.

Altered MAP2 immunoreactivity may well be related to the diminished arborization of subicular apical dendrites that we have observed in schizophre-

nia [6]. Such an association occurs in Rett syndrome, where both dendritic branching [49] and MAP2 immunoreactivity [50] are diminished. In cultured neurons, MAP2 levels are proportional to the degree of dendritic branching [51]. A related possibility is a decreased frequency of synaptic remodeling, which is normally accompanied by a transient increase in MAP2 immunoreactivity [30, 52, 53].

MAP2 immunoreactivity is decreased in subiculum following serotonin depletion [54, 55] and in neocortex following lesion of the ipsilateral nucleus basalis of Meynert, the main source of extrinsic cholinergic input [28]. An increase in MAP2 immunoreactivity of hippocampal CA1 pyramidal cell bodies occurs after lesion of dopaminergic cells of the ventral tegmental area [27]. Thus, in schizophrenia, the loss of MAP2 immunoreactivity may result from abnormal neurotransmitter activity caused by the disease or its treatment.

Altered MAP2 immunoreactivity could be related to altered activity of NMDA receptors [56, 57, 58]. Diminished stimulation of NMDA receptors would theoretically lead to increased phosphorylation of MAP2 [56, 58]. Increased phosphorylation would cause decreased association of MAP2 with microtubules and neurofilaments, which could lead to diminished immunoreactivity [59]. Conversely, since the effects of NMDA receptors are in part mediated by phosphorylation of MAP2, a loss or alteration of MAP2 could lead to decreased effectiveness of the receptors. Since the effect of NMDA receptors on MAP2 dephosphorylation is much greater in the adult than in the neonate [58], such a mechanism could account for the age of onset of schizophrenia. This mechanism would also be consistent with developmentally regulated expression of various MAP2 isoforms [12], coded by differential splicing of a single gene [60]. Perturbation of this regulation in subicular neurons could lead to expression of illness at a particular point in development, with diminished expression of MAP2a or MAP2b accounting for the loss of immunoreactivity.

Conclusion

This study supports other reports of alterations of subicular MAP2 in schizophrenia and indicates that these are not associated with gliosis. The effects of phosphorylation, differential splicing, binding to microtubules, post mortem delay, and fixation on the immunoreactivity of different MAP2 epitopes are too complex to allow any conclusions about what is happening to MAP2 on a cellular level. These uncertainties may explain some of the differences among studies, and they clearly indicate the need for biochemical studies in addition to immunohistochemistry. The critical neuroanatomic location, the importance of MAP2 to dendritic structure, and the association of altered immunoreactivity with a course characterized by fewer symptoms suggest an important functional role for MAP2 in schizophrenia.

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Резиме

НАРУШЕНА СУБИКУЛАРНА ИМУНОРЕАКТИВНОСТ НА MAP2 КАЈ ШИЗОФРЕНИЈАТА

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Цел на овј труд е да се направи независна анализа на претходно публикуваните резултати за постоење загуба во регијата на субикулумот, на протеинот асоциран со микротубулите (MAP2) во мозокот на индивидуи што страдале од шизофренија, како и да се види дали постои корелација меѓу клиничката слика и степенот на неговата загуба.

За таа цел, направивме имунохистохемиска анализа во регијата на хипокампусот кај 94 психијатриски пациенти, од кои 64 со примарна дијаг-

ноза шизофренија или шизоафективно растројство, 12 со примарна дијагноза мајорна депресија или биполарно растројство, 18 со примарна дијагноза деменција, како и кај 17 индивидуи без психијатриска дијагноза. Психијатриската симптоматологија што индивидуите ја имале во тек на животот беше евалуирана со инструмент наречен: *Модифицирана дијагностичка евалуација по смртта*.

Имунореактивноста на MAP2 во субикулумот беше значително намалена кај 20% од шизофрениите, кај 8% од афективните растројства и кај 22% од деменциите. Кај сите индивидуи без психијатриска дијагноза имуноекспресијата на MAP2 беше нормална. Кај деменциите, оние со намалена имуноекспресија на MAP2 имаа поназначена глиоза во субикулумот, додека кај шизофрениите не постоеше ваква асоцијација.

Кај шизофрениите, губитокот на субикуларната имунореактивност за MAP2 беше асоцирана со редуцирање на позитивните и негативните симптоми во текот на болеста.

Субикуларната MAP2 е забележливо редуцирана кај значителен процент индивидуи што се хронично хоспитализирани заради шизофрено растројство, без постоење генерализирана деструкција на субикуларните неурони. За разлика од нив, кај индивидуите хоспитализирани заради деменција, губитокот на MAP2 е придружен од глиоза.

Губитокот на MAP2 е асоциран со помал број клинички симптоми, што сугерира дека оваа загуба може да претставува адаптивен одговор на мозокот кон шизофреното заболување.

Хемиските и структурните абнормалности што се во основа на намалената MAP2 имунореактивност кај шизофренијата треба понатаму да се иследуваат.

Клучни зборови: дендрит, шизофренија субикулум MAP2. Невронаучни кодови: пореметување на нервниот систем, невропсихијатриски пореметувања.

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Immunoreactivity for GFAP

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