Archival Report

Biological Psychiatry

Loss of Microtubule-Associated Protein 2 Immunoreactivity Linked to Dendritic Spine Loss in Schizophrenia

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ABSTRACT

BACKGROUND: Microtubule-associated protein 2 (MAP2) is a neuronal protein that plays a role in maintaining dendritic structure through its interaction with microtubules. In schizophrenia (Sz), numerous studies have revealed that the typically robust immunoreactivity (IR) of MAP2 is significantly reduced across several cortical regions. The relationship between MAP2-IR reduction and lower dendritic spine density, which is frequently reported in Sz, has not been explored in previous studies, and MAP2-IR loss has not been investigated in the primary auditory cortex (Brodmann area 41), a site of conserved pathology in Sz.

METHODS: Using quantitative spinning disk confocal microscopy in two cohorts of subjects with Sz and matched control subjects (Sz subjects, $n = 20$; control subjects, $n = 20$), we measured MAP2-IR and dendritic spine density and spine number in deep layer 3 of BA41.

RESULTS: Subjects with Sz exhibited a significant reduction in MAP2-IR. The reductions in MAP2-IR were not associated with neuron loss, loss of MAP2 protein, clinical confounders, or technical factors. Dendritic spine density and number also were reduced in Sz and correlated with MAP2-IR. In 12 (60%) subjects with Sz, MAP2-IR values were lower than the lowest values in control subjects; only in this group were spine density and number significantly reduced.

CONCLUSIONS: These findings demonstrate that MAP2-IR loss is closely linked to dendritic spine pathology in Sz. Because MAP2 shares substantial sequence, regulatory, and functional homology with MAP tau, the wealth of knowledge regarding tau biology and the rapidly expanding field of tau therapeutics provide resources for identifying how MAP2 is altered in Sz and possible leads to novel therapeutics.

Keywords: Schizophrenia, Microtubule-Associated Protein 2, Dendritic spines, Auditory cortex, Postmortem human tissue, Confocal microscopy

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Individuals with schizophrenia (Sz) present with numerous functional deficits in the auditory domain. Patients exhibit impaired performance on pure-tone discrimination tasks, an inability that does not depend on attention and implicates the auditory cortex itself (1–3). This functional deficit has consequences for social cognition in individuals with Sz in that it makes prosody detection more difficult (4,5). In electrophysiologic studies, subjects with Sz display reduced amplitude on mismatch negativity (1,6–8), an event-related potential generated in the primary auditory cortex in response to stimuli deviant from preceding stimuli with respect to a particular feature (e.g., pitch, amplitude, duration) (9). Performance on tone discrimination tasks and mismatch negativity amplitude are correlated, and impairments on both are linked to severity of positive and negative symptoms (1,3,10).

These functional and electrophysiologic deficits are paralleled at the cortical level by progressive gray matter volume reduction in the superior temporal gyrus in subjects with Sz and first-degree relatives at high risk (11,12) and specifically in Heschl's gyrus, which contains the primary auditory cortex (12–16) (Brodmann area [BA] 41). Reductions in gray matter are found at (12,16), or before transition to (13–15), the first psychotic episode indicating initial gray matter loss cannot be attributed to the effects of treatment or illness duration. Superior temporal gyrus gray matter loss is selective for individuals with Sz compared with individuals diagnosed with bipolar disorder (13–15).

Gray matter volume loss in the auditory cortex in Sz is not explained by underlying reductions in layer 3 pyramidal neuron number (17) and more likely represents reductions in pyramidal neuron somal size and excitatory connections in this cortical region (18,19). We previously described a decrease in the density of spinophilin-immunoreactive puncta in deep layer 3 of the primary auditory cortex in Sz, representing reductions in

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pyramidal neuron spine density paralleling findings of a loss of spines per length of dendrite described in other cortical regions (20–22). Also, Sz is characterized by a reduction in the extent and complexity of the dendritic arbor in hippocampus and cingulate and frontal cortices (21,23–25). However, the molecular mechanisms that contribute to these concurrent reductions in dendrites and spines in disease are currently unknown.

Microtubule-associated protein 2 (MAP2) stands at the intersection of these phenomena. MAP2 is the most prevalent isoform of the dendritic MAPs, a family of cytoarchitectural proteins that includes the axonal homologue MAP tau (26,27). MAP2 is an important regulator of neuritic development and maintenance that acts by binding and nucleating the primary structural component of the dendritic cytoskeleton, microtubule (MT) monomers, and subsequently stabilizing and spacing mature MT bundles in the dendrite (26–30). MAP2 plays a similar role in supporting the actin cytoskeleton in spines, binding and nucleating filamentous actin to regulate spine morphology (31). MAP2 is regulated by development and experience-dependent plasticity, with these processes tightly controlling MAP2 function by phosphorylation across its functional domains (27). Immunoreactivity of MAP2 (MAP2-IR) is markedly reduced in many different cortical regions associated with Sz pathology, including regions where reduction of the dendritic arbor has been described (32–36) (e.g., cingulate and frontal cortices, hippocampal formation). However, MAP2 messenger RNA expression levels are unchanged in the disorder suggesting that Sz pathology affects MAP2 protein and not its transcript (37).

In the present study, we investigated whether MAP2-IR is diminished in deep layer 3 of the primary auditory cortex of subjects with Sz and its potential association with spine reduction, which we previously observed in this layer (18). To address this question, we used multilabel quantitative fluorescence microscopy to measure the intensity of MAP2-IR, spine density, and spine number in 20 subjects with Sz and matched control subjects. We found that MAP2-IR was significantly decreased in individuals with Sz, with a subset of 60% of subjects with Sz that exhibited MAP2-IR levels below the lowest level observed in control subjects. MAP2-IR was significantly associated with spine density and spine number, with reductions in spine density and number restricted to the 60% of subjects with Sz with MAP2-IR below normal levels. These findings suggest that MAP2 is functionally compromised by disease pathology with implications for dendritic arbor and dendritic spine structural integrity.

METHODS AND MATERIALS

Human Subjects and Animals

For this study, we used tissue from two cohorts (Table 1) consisting of subjects with a diagnosis of Sz or schizoaffective disorder (together referred to as Sz) and control subjects matched on the basis of sex and as closely as possible for age, postmortem interval (PMI), and handedness. We also used a previously described monkey (*Macaca fascicularis*) cohort consisting of four animals chronically administered the antipsychotic haloperidol decanoate and control animals

Table 1. Subject Characteristics

	Cohort 1		Cohort 2		Total	
	Control	Sz	Control	Sz	Control	Sz
n	12	12	8	8	20	20
Mean Age, Years (SD)	45.2 (12.9)	47.3 (13.4)	46.4 (14.0)	46.5 (12.4)	45.8 (13.0)	46.9 (13.4)
Range	19–65	27–71	24–62	25–62	19–65	25–71
Sex (F/M)	3/9	3/9	4/4	4/4	7/13	7/13
Handedness (R/L/A/U)	11/1/0/0	6/2/1/3	8/0/0/0	5/3/0/0	19/1/0/0	11/5/1/3
PMI (SD)	18.1 (6.5)	17.9 (8.8)	13.7 (6.5)	15.6 (6.8)	16.4 (6.7)	17.0 (7.9)
Storage Time, Months (SD)	155.0 (27.2)	145.5 (29.8)	97.1 (22.4)	92.8 (14.0)	131.8 (38.2)	124.4 (35.9)
Illness Duration, Years (SD)		22.1 (14.7)		22 (13.3)		22.1 (13.8)
Range		3–50		4–41		3–50
Age at Onset, Years (SD)		25.2 (7.7)		24.5 (9.6)		24.9 (8.3)
Suicide, *n* (%)		2 (16.7%)		2 (25.0%)		4 (20.0%)
Schizoaffective, *n* (%)		4 (33.3%)		2 (25.0%)		6 (30.0%)
Alcohol/Substance Abuse ATOD		5 (41.7%)		0 (0%)		5 (25.0%)
Anticonvulsant ATOD, *n* (%)		5 (62.5%)		1 (12.5%)		6 (30.0%)
Antidepressant ATOD, *n* (%)		3 (37.5%)		5 (62.5%)		8 (40.0%)
Antipsychotic ATOD, *n* (%)		11 (91.7%)		6 (75.0%)		17 (85.0%)
Benzodiazepine ATOD, *n* (%)		1 (8.3%)		3 (37.5%)		4 (20.0%)
History of Cannabis Use, *n* (%)		5 (41.7%)		2 (25.0%)		7 (35.0%)
Tobacco ATOD, *n* (%)	4 (33.3%)	8 (66.7%)	3 (37.5%)	6 (75.0%)	7 (35.0%)	14 (70.0%)

Each Sz subject in cohorts 1 and 2 was previously matched to a normal control subject based on sex and as closely as possible for age and postmortem interval. There were no diagnostic group differences in age [$t_{38} = -.333$, $p = .741$] or postmortem interval [$t_{38} = -.272$, $p = .787$]. The distribution of handedness between diagnostic groups reached trend level ($\chi^2 = 8.800$, $p = .066$). Mean tissue storage time did not differ between diagnostic groups (cohort 1 [$t_{22} = .817$, $p = .423$], cohort 2 [$t_{14} = .461$, $p = .652$]).

A, ambidextrous; ATOD, at time of death; F, female; L, left-handed; M, male; PMI, postmortem interval; R, right-handed; SD, standard deviation; Sz, schizophrenia; U, unknown.