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# Increased stability of microtubules in cultured olfactory neuroepithelial cells from individuals with schizophrenia

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ABSTRACT

Microtubules (MTs) are essential components of the cytoskeleton that play critical roles in neurodevelopment and adaptive central nervous system functioning. MTs are essential to growth cone advance and ultrastructural events integral to synaptic plasticity; these functions figure significantly into current pathophysiologic conceptualizations of schizophrenia. To date, no study has directly investigated MT dynamics in humans with schizophrenia. We therefore compared the stability of MTs in olfactory neuroepithelial (OE) cells between schizophrenia cases and matched nonpsychiatric comparison subjects. For this purpose, we applied nocodazole (Nz) to cultured OE cells obtained from tissue biopsies from seven living schizophrenia patients and seven matched comparison subjects; all schizophrenia cases were on antipsychotic medications. Nz allows MT depolymerization to be followed but prevents repolymerization, so that in living cells treated for varying time intervals, the MTs that are stable for a given treatment interval remain. Our readout of MT stability was the time at which fewer than 10 MTs per cell could be distinguished by anti-β-tubulin immunofluorescence. The percentage of cells with ≥10 intact MTs at specified intervals following Nz treatment was estimated by systematic uniform random sampling with Visiopharm software. These analyses showed that the mean percentages of OE cells with intact MTs were significantly greater for schizophrenia cases than for the matched comparison subjects at 10, 15, and 30 min following Nz treatment indicating increased MT stability in OE cells from schizophrenia patients ($p = 0.0007$ at 10 min; $p = 0.0008$ at 15 min; $p = 0.036$ at 30 min). In conclusion, we have demonstrated increased MT stability in nearly all cultures of OE cells from individuals with schizophrenia, who received several antipsychotic treatments, versus comparison subjects matched for age and sex. While we cannot rule out a possible confounding effect of antipsychotic medications, these findings may reflect analogous neurobiological events in at least a subset of immature neurons or other cell types during gestation, or newly generated cells destined for the olfactory bulb or hippocampus, suggesting a mechanism that underlies findings of postmortem and neuroimaging investigations of schizophrenia. Future studies aimed at replicating these findings, including samples of medication-naïve subjects with schizophrenia, and reconciling the results with other studies, will be necessary. Although the observed abnormalities may suggest one of a number of putative pathophysiologic anomalies in schizophrenia, this work may ultimately have implications for an improved understanding of pathogenic processes related to this disorder.



*Abbreviations:* MTs, microtubules; OE, olfactory neuroepithelium; Nz, nocodazole; NMDA, N-methyl-D-aspartate; MAPs, microtubule associated proteins; DISC1, disrupted-in-schizophrenia-1; DLPFC, dorsolateral prefrontal cortex; NUDEL, nuclear distribution protein nude-like 1; NAA, N-acetylaspartate; Cr, creatine; Nz, nocodazole; SCID-P, Structured Clinical Interview for the Diagnostic and Statistical Manual of Mental Disorders, 4th ed., Patient Edition; HBSS, Hank's Balanced Salt Solution; DMEM, Dulbecco's Modified Eagle's Medium; FBS, Fetal Bovine Serum; EDTA, ethylenediaminetetraacetic acid; PBS, Phosphate-Buffered Saline; DMSO, dimethyl sulfoxide; DAPI, 4′, 6-diamidino-2-phenylindole; GFAP, glial fibrillary acidic protein; +TIPs, plus-end-tracking-proteins; DF, degrees of freedom; ADNP, activity-dependent neuroprotective protein (ADNP).

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## 1. Introduction

Microtubules (MTs) are hollow cylindrical filaments polymerized from α/β-tubulin heterodimers. As essential components of the cytoskeleton, MTs play critical roles in various neurodevelopmental processes and adaptive central nervous system functioning (Dent and Gertler, 2003; Dent et al., 2011; Hoogenraad and Akhmanova, 2010; Rodriguez et al., 2003). In developing neurons, MTs steer growth cones as they interact with actin filaments contributing to growth-cone advance and turning in filopodia, and consequently govern axon guidance (Geraldo and Gordon-Weeks, 2009).

MTs are also critical for ultrastructural changes that are integral to the development of synaptic plasticity (Jaworski et al., 2009). While stable MTs do not undergo polymerization–depolymerization (Bulinski and Gundersen, 1991) and are confined to the shafts of dendrites, dynamic MTs enter dendritic spines, where they regulate levels of F-actin, maintaining the structure of individual spines and their synapses. Thus, modulation of MT dynamics can affect and remodel ultrastructural components of synapses.

During neurodevelopment, the roles of MTs figure significantly into current pathophysiologic theories of schizophrenia (Balu and Coyle, 2011; Greer and Greenberg, 2008; Rosoklija et al., 2000). Some postmortem studies have suggested deficits of neuronal migration and outgrowth of axons and dendrites, two processes that depend, in part, on MTs (Balu and Coyle, 2011; Falkai et al., 2000). The N-methyl-D-aspartate (NMDA) receptor hypofunction hypothesis of schizophrenia is supported by numerous investigations and can account for multiple symptom domains of this disorder (Greer and Greenberg, 2008; Wayman et al., 2008). NMDA receptors are located primarily on dendritic spines, whose size and shape are important determinants of the effects of activated receptors. In turn, activation of NMDA receptors increases MTs in the dendritic spines and increases their size (Merriam et al., 2011). NMDA receptor hypofunction can thus lead to altered MT dynamics as well as decreased dendritic spine densities as found in neocortex and subiculum of schizophrenia cases (Balu and Coyle, 2011; Garey et al., 1998; Glantz and Lewis, 2000; Rosoklija et al., 2000).

Altered MT dynamics represent a potential mechanism by which a mutation in the disrupted-in-schizophrenia-1 (*DISC1*) gene has been hypothesized to contribute to schizophrenia (Kamiya et al., 2005). The product of the DISC1 gene, in which a disruption by a translocation is strongly associated with schizophrenia and other psychiatric disorders in a large Scottish pedigree, interacts with proteins that affect centrosomal and MT function, and may affect MT dynamics; these proteins include NUDEL, and the microtubule-associated proteins (MAPs), MIP-T3 and MAP1A (Morris et al., 2003). DISC1 is a component of the microtubule-associated dynein motor complex (with dynein, dynactin, NUDEL, and LIS1) and appears to be essential for maintaining the complex at the centrosome and for normal neuronal migration and formation of axons (Kamiya et al., 2005).

MAPs, which bind to MTs but not to unpolymerized tubulin, act as MT stabilizers and influence axon guidance. Among these is the stable tubule only polypeptide (STOP, or MAP6), which is proposed to have a role in regulating MT dynamics by imparting cold-stability to MTs (Andrieux et al., 2002). Interestingly, mice with a loss-of-function mutation of this gene exhibit synaptic hippocampal, neurotransmitter, and behavioral abnormalities analogous to those found in schizophrenia (Andrieux et al., 2002; Brun et al., 2005; Fradley et al., 2005). Most strikingly, the mothers fail to feed their pups, and this behavior is reversed by chronic, but not acute, treatment with antipsychotic medications (Andrieux et al., 2002; Brun et al., 2005). Davunetide (NAP), an 8-amino acid peptide derived from activity-dependent-neuroprotective protein (ADNP), interacts with MTs to promote neurite outgrowth and synaptogenesis. Interestingly, this medication diminished hyperlocomotion and improved object recognition/discrimination and spatial memory in the STOP heterozygous mouse (Merenlender-Wagner et al., 2010). Epothilone D, a MT stabilizer, was shown to reverse abnormalities in STOP-null mice, including increasing synaptic vesicle density, improving long-term potentiation, and markedly improving maternal behavior and pup survival (Andrieux et al., 2006).

In a 12-week multi-center double blind clinical trial, davunetide was related to significant improvement in the UCSD Performance Based Skills Assessment (UPSA) (Javitt et al., 2012), which is considered to measure functionally meaningful cognition. In addition, davunetide led to a trend-level increase in the N-acetylaspartate (NAA)/creatine (Cr) ratio and a significant increase in the choline/Cr ratio in dorsolateral prefrontal cortex (DLPFC) in schizophrenia (Jarskog et al., 2013), suggesting neurotrophic or neuroprotective effects. In culture, davunetide increased the MT network area, had neurotrophic effects, and protected cells against tubulin and tau loss from assembled MT related zinc toxicity (Oz et al., 2012).

Notwithstanding the important findings in mice, studies of STOP in humans have not been consistent with this model. For example, a postmortem brain study of DLPFC showed that mRNA levels of two STOP isoforms were *increased* in schizophrenia (Shimizu et al., 2006). A proteomic analysis also revealed increased STOP levels, as well as increased MAP2 and MAP1A in the DLPFC (Martins-de-Souza et al., 2009) in schizophrenia. Increased MAP2 levels have also been found in hippocampus (Cotter et al., 2000), while normal levels of MAP2 were found in cerebellum in schizophrenia cases (Mukaetova-Ladinska et al., 2002). These findings would predict increased stability of MTs in patients with schizophrenia. Notably, however, studies from other laboratories have reported decreased MAP2 expression in hippocampus and prefrontal cortex (Arnold et al., 1991; Rioux et al., 2004; Somenarain and Jones, 2010), and thus the question of MT stability in schizophrenia patients has not been resolved.

To date, no study has directly investigated MT dynamics or stability in humans with schizophrenia. Studies of the dynamics of MTs require live neurons from patients, posing daunting ethical constraints. Accordingly, we approached this problem by employing olfactory neuroepithelium (OE), which can be obtained by biopsies that are very safe and efficient. The regenerative neuroepithelial tissue contains pluripotent cells, from which in vitro neuroepithelial cells can be propagated with little modification of genomic or epigenomic profiles. As such, these cells are more likely to harbor in vivo neurobiological characteristics of donors compared to neurons derived from pluripotent stem cells induced to form neurons (Pang et al., 2011), which require the expression of appropriate transcription factors. Solis-Chagoyan et al. (2013) have utilized OE to evaluate MT organization in schizophrenia cases and controls.

The application of nocodazole (Nz), a synthetic compound that prevents the polymerization of tubulin, allows for direct assessment of MT stability. For this initial study, we chose the Nz resistance assay as it would allow for the measurement of MT stability both qualitatively (increase or decrease in stability) and quantitatively (a modest or more dramatic change in stability). Since Nz blocks repolymerization of tubulin, living cells can be treated for varying time intervals to assess the degree of stability of cellular MTs (Piperno et al., 1987). Here, we applied Nz to cultured cells harvested from OE of live individuals with and without schizophrenia in order to directly compare the stability of their MTs. We hypothesized that there would be increased stability of MTs in patients with schizophrenia.

## 2. Methods

### 2.1. Subjects

Individuals aged 18–55 were recruited through the Schizophrenia Research Center at the University of Pennsylvania. Consensus diagnoses of schizophrenia were determined based on interview with the Structured Clinical Interview for the *Diagnostic and Statistical Manual of Mental Disorders, 4th ed., Patient Edition* (SCID-P). Non-psychiatric comparison subjects were interviewed with the SCID, Non-Patient Edition and were determined to be free of any Axis I or Axis II cluster A (i.e.