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Structural and functional changes in tau mutant mice neurons are not linked to the presence of NFTs

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ABSTRACT

In the rTg4510 mouse model, expression of the mutant human tau variant P301L leads to development of neurofibrillary tangles (NFTs), neuronal death, and memory impairment, reminiscent of the pathology observed in human tauopathies. In the present study, we examined the effects of mutant tau expression on the electrophysiology and morphology of individual neurons using whole-cell patch-clamp recordings and biocytin filling of pyramidal cells in cortical slices prepared from rTg4510 (TG) and wild-type (WT) littermate mice. Among the TG cells, 42% contained a clear Thioflavin-S positive inclusion in the soma and were categorized as NFT positive (NFT+), while 58% had no discernable inclusion and were categorized as NFT negative (NFT−). The resting membrane potential (V_r) was significantly depolarized (+8 mV) in TG cells, and as a consequence, evoked repetitive action potential (AP) firing rates were also significantly increased. Further, single APs were significantly shorter in duration in TG cells and the depolarizing voltage deflection or "sag" evoked by hyperpolarization was significantly greater in amplitude. In addition to these functional electrophysiological changes, TG cells exhibited significant morphological alterations, including loss or significant atrophy of the apical tuft, reduced dendritic complexity and length, and reduced spine density. Importantly, NFT− and NFT+ TG cells were indistinguishable with regard to both morphological and electrophysiological properties. Our observations show that expression of mutated tau results in significant structural and functional changes in neurons, but that these changes occur independent of mature NFT formation.

Introduction

The microtubule-associated protein tau plays a key role in stabilization and assembly of microtubules, which are critical for the maintenance of normal cellular morphology and cellular trafficking. Neurodegenerative tauopathies are characterized by abnormal intracellular accumulations of fibrillar tau, termed neurofibrillary tangles (NFTs), which can result from mutation of the tau gene (e.g. frontotemporal dementia and parkinsonism linked to chromosome 17; FTDP-17), mutation of other disease-related genes such as the one encoding amyloid precursor protein (e.g. Alzheimer's Disease), or from currently undefined mechanisms (review: Lee et al., 2001; Ballatore et al., 2007). Tau mutations result in decreased binding of tau to microtubules, resulting in destabilization of microtubules; unbound tau then develops a propensity to hyperphosphorylate, recompartmentalize from the axon to the soma and dendrites, and aggregate in the form of NFTs. The P301L mutation of tau is commonly seen in FTDP-17 (Poorkaj et al., 2001), and the conditional mouse model rTg4510 that expresses the P301L mutation closely mimics features of human tauopathies including progressive NFT deposition, widespread neuron death, and memory impairment (Santacruz et al., 2005).

Studies examining the relationship between tau and neuronal death in specific brain regions have resulted in varying conclusions regarding the role that NFTs versus soluble non-fibrillar species play in neurodegeneration (review: Bretteville and Planel, 2008; Congdon and Duff, 2008; Spires-Jones et al., 2009). The number of NFTs has been positively correlated with the progression of neurodegeneration and dementia in AD (Lee et al., 2001) leading to the widely-held assumption that NFTs are neurotoxic. However, data from several studies suggest a possibly neutral, if not outright protective, role for NFTs (Bussière et al., 2003; Bretteville and Planel, 2008; Congdon and Duff, 2008). For example, in mice that overexpress wild-type human tau, extensive neuronal death occurred prior to substantial deposition of aggregated tau and NFTs, and neurons that appeared to be dying exhibited no ultrastructural evidence of fibrillar tau (Andorfer et al., 2005). Similarly, it has been demonstrated that NFTs and neuronal death are not associated in the hippocampus, cortex, or striatum of the rTg4510 mouse model (Spires et al., 2006). While these studies have assessed the relationship of NFTs to neuronal death, information regarding the effect of NFTs and of soluble non-fibrillar tau on the structure and function of individual neurons is lacking. Since tau

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interacts with a wide array of structural and functional proteins, mutations in tau that lead to NFT deposition could be expected to significantly alter neuronal structure and function without necessarily being lethal to a given neuron. Because cellular morphology is a primary determinant of neuronal firing patterns and synaptic integration, it is logical to further hypothesize that any tau-related morphological changes may lead to alterations in functional electrophysiological properties which, in turn, could impact neuronal network properties in a given brain area. In the present study, the effects of tau mutation on the structure and function of individual frontal cortical neurons in rTg4510 versus wild-type mice were assessed for the first time.

Materials and methods

Experimental subjects

Studies were performed using six rTg(tau_{P301L})4510 (TG) and six age-matched wild-type (WT) mice that were 8.5 months of age. Mice were screened for activator and responder transgenes (Santacruz et al., 2005) using a standardized PCR assay for tail DNA. All mice were given *ad libitum* access to food and water. Animal care and experiments were conducted in accordance with standards set forth by the National Institutes of Health Guide for the Care and Use of Laboratory Animals and the United States Public Health Service Policy on Humane Care and Use of Laboratory Animals. The Mayo Clinic and the Boston University Institutional Animal Care and Use Committees (IACUC) approved all animal procedures.

Slice preparation

Mice were sacrificed by decapitation and their brains were rapidly removed and submerged in oxygenated (95% O_2 and 5% CO_2) ice-cold Ringer's solution, concentrations (in mM): 25 $NaHCO_3$, 124 NaCl, 1 KCl, 2 KH_2PO_4, 10 Glucose, 2.5 $CaCl_2$, and 1.3 $MgCl_2$, (pH 7.4; Sigma-Aldrich, St. Louis, MO). Post-extraction, dissected cortical hemispheres were affixed to an agar slab with cyanoacrylate glue and placed in a tissue holder for cutting. Eight to 10 coronal slices (300 μm thick) of the rostral third of the brain were cut into ice-cold Ringer's solution with a vibrating microtome. Following cutting, slices were equilibrated for a minimum of 1 h at room temperature (RT) in oxygenated Ringer's solution. For recording, individual slices were positioned in submersion-type recording chambers (Harvard Apparatus, Holliston, MA) on Nikon E600 infrared-differential interference contrast microscope (IR-DIC; Micro Video Instruments, Avon, MA) stages and were continuously perfused with RT oxygenated Ringer's solution (2–2.5 ml/min).

Whole-cell patch-clamp recordings

Whole-cell patch-clamp recordings were obtained from individual layer 3 frontal cortical pyramidal cells which were identified under IR-DIC optics. For assessment of electrophysiological properties, recordings were conducted as previously described (Chang and Luebke, 2007; Chang et al., 2005). Patch electrode pipettes pulled from capillary tubes on a Flaming and Brown horizontal pipette puller (Model P87, Sutter Instrument, Novato, CA) were filled with potassium methanesulfonate internal solution, concentrations (in mM): 122 KCH_3SO_3, 2 $MgCl_2$, 5 EGTA, and 10 NaHEPES containing 1% biocytin (pH 7.4; Sigma-Aldrich, St. Louis, MO). In Ringer's solution, pipettes had a resistance of between 3 and 6 MΩ. "PatchMaster" acquisition software (HEKA Elektronik, Lambrecht, Germany) was used for data acquisition with EPC-9 and EPC-10 amplifiers (HEKA Elektronik). Signals were low-pass filtered at 10 kHz. Cells were maintained at resting membrane potential during current-clamp recordings.

Passive membrane properties and action potential (AP) firing properties

Resting membrane potential (V_r), input resistance (R_n) and membrane time constant were determined in current-clamp mode. V_r was determined as the voltage recorded in the absence of current injection. R_n was calculated as the slope of a best-fit line through a voltage–current relationship plot of steady-state voltage responses to 9 successive 10 pA current steps (−40 to +40 pA), 200 ms in duration. Membrane time constant was determined by fitting a single-exponential function to the first 100 ms of a 200 ms, 20 pA hyperpolarizing current step. Single AP property measurements were performed on the first AP generated in a 200 ms current-clamp series. These properties included AP threshold, amplitude, and duration at half amplitude. The rheobase (minimum current required to evoke a single AP), was determined with a 10 s depolarizing current ramp from 0–200 pA using the FitMaster software (HEKA Elektronik) measuring tool. AP firing threshold was measured on an expanded time scale at the initiation of the sharp upward spike deflection. AP amplitude was calculated from the threshold to the peak of the spike and duration at half amplitude of the AP. A series of 12 hyperpolarizing and depolarizing current steps (in 50 pA increments from −170 to +380 pA), each 2 s in duration, was implemented for evaluation of repetitive AP firing. Characteristic of an I_h mediated depolarization (Zhang et al., 2006), the 2 s −170 pA hyperpolarizing current step evoked a slowly emergent depolarizing voltage "sag". "Sag" amplitude was measured from the minimum voltage to the maximum amplitude of deflection toward depolarization using the FitMaster software measuring tool. FitMaster software event detection was used for determination of AP frequency.

Slice processing and confocal scanning

During recording (~15 min/cell), cells were simultaneously filled with biocytin. Slices were then promptly placed in a 4% paraformaldehyde in 0.1 M phosphate buffered saline (PBS) solution (pH 7.4). Following overnight fixation at 4 °C, slices were rinsed in PBS (3 times, 10 min each) and placed in 0.1% Triton X-100/PBS for 2 h at RT. Slices were then incubated in streptavidin–Alexa 546 (1:500; InVitrogen, Carlsbad, CA) at 4 °C for 48 h. For visualization of neurofibrillary tangles (NFTs) slices from both groups (WT and TG) were incubated in 0.01% Thioflavin-S. Thioflavin-S staining is a sensitive method conventionally employed for the post-mortem detection of NFTs in brains from patients with tauopathy (e.g. Sun et al., 2002; Santa-Maria et al., 2006) and from animal models of tauopathy (review: Lewis and Hutton, 2005). Following mounting with Prolong Gold mounting medium (InVitrogen) and coverslipping, stained slices were scanned with a Zeiss 510 confocal laser-scanning microscope using a Plan-Apochromat 40×/1.3 NA oil objective lens (210 μm working-distance). For localization of NFTs in somata, fluorescence emitted by Alexa-546 (Helium/Neon laser excitation) and Thioflavin-S (Argon laser excitation) were collected in two channels with 560 nm and 480–520 nm pass filters respectively. Each "soma stack" was acquired at a resolution of 0.1×0.1×0.5 μm per voxel (Fig. 1). For dendritic reconstructions, two to three stacks per cell were obtained using a voxel size of 0.4×0.4×0.41 μm. Approximately 10 stacks per cell, each with a field of view of 153 μm^2, were acquired at a very high resolution for spine analyses (5 cells per group). These stacks were captured using a 0.1×0.1×0.2 μm voxel size.

Data processing and three-dimensional (3D) morphologic analyses

Pre-processing of image stacks

Each image stack was deconvolved using Autodeblur software (Media Cybernetics, Bethesda, MD) to reduce signal blurring. With Volume Integration and Alignment System (VIAS) software (Rodriguez et al., 2003), deconvolved stacks were aligned in 3D and