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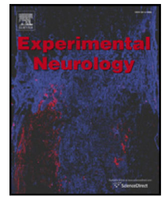

Regular Article

Selective impact of Tau loss on nociceptive primary afferents and pain sensation

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ABSTRACT

Tau protein hyperphosphorylation and consequent malfunction are hallmarks of Alzheimer's disease pathology; importantly, pain perception is diminished in these patients. In physiological conditions, Tau contributes to cytoskeletal dynamics and in this way, influences a number of cellular mechanisms including axonal trafficking, myelination and synaptic plasticity, processes that are also implicated in pain perception. However, there is no in vivo evidence clarifying the role of Tau in nociception. Thus, we tested Tau-null (Tau −/−) and Tau +/+ mice for acute thermal pain (Hargreaves' test), acute and tonic inflammatory pain (formalin test) and mechanical allodynia (Von Frey test). We report that Tau −/− animals presented a decreased response to acute noxious stimuli when compared to Tau +/+ while their pain-related behavior is augmented under tonic painful stimuli. This increased reactivity to tonic pain was accompanied by enhanced formalin-evoked c-fos staining of second order nociceptive neurons at Tau-null dorsal horn. In addition, we analyzed the primary afferents conveying nociceptive signals, estimating sciatic nerve fiber density, myelination and nerve conduction. Ultrastructural analysis revealed a decreased C-fiber density in the sciatic nerve of Tau-null mice and a hypomyelination of myelinated fibers (Aδ-fibers) – also confirmed by western blot analysis – followed by altered conduction properties of Tau-null sciatic nerves. To our knowledge, this is the first in vivo study that demonstrates that Tau depletion negatively affects the main systems conveying nociceptive information to the CNS, adding to our knowledge about Tau function(s) that might also be relevant for understanding peripheral neurological deficits in different Tauopathies.

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Introduction

Tau malfunction, through its abnormal hyperphosphorylation, is postulated as a crucial mechanism of Alzheimer's disease (AD) neuronal dysfunction but it remains unknown whether Tau protein could be a direct or indirect contributor to altered pain processing found in AD patients (Corbett et al., 2012). The predominant view about Tau function focuses on its key cytoskeletal role based on its ability to bind to microtubules (MT) and other cytoskeletal elements (Mandelkow and Mandelkow, 2012). Interestingly, neuropathic pain is a common side effect of MT-targeting agents commonly used in the clinics as chemotherapeutic drugs (e.g. vincristine, paclitaxel) (Jaggi and Singh, 2012). In addition, a growing body of evidence suggests that Tau has an important role in the dendritic compartment where, via interaction with Fyn, contributes to NMDA receptor/PSD95 complex formation (Frandemiche et al., 2014; Ittner and Gotz, 2011) that has an established role in pain-related sensitization (D'Mello et al., 2011). There is also direct evidence of Fyn involvement on pain sensitization through phosphorylation of the NMDA NR2B subunit (Abe et al., 2005). Furthermore, Fyn constitutional activation results in hyperalgesia in naive mice (Liu et al., 2014). Moreover, a cell culture-based study suggests that Tau–Fyn interaction may be involved in myelination regulation (Klein et al., 2002), which is of relevance for optimal axonal function and, consequently, for nociceptive transmission. Furthermore, there is also evidence that other cytoskeletal elements may be involved in pain as disruptors of microtubules, actin filaments and neurofilaments in primary afferent nociceptors interfere with normal pain sensation (Reichling and Levine, 2011) reinforcing the importance of understanding the relation between cytoskeletal proteins and pain.

Abbreviations: AD, Alzheimer's disease; NMDA, N-methyl-D-aspartate; CNS, central nervous system; PNS, peripheral nervous system; MT, microtubules; MBP, myelin basic protein.

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An initial step for pain perception is the activation of peripheral nociceptors which generates a local depolarization conveyed by specific fibers to secondary afferent neurons in the dorsal horn. Responding to mechanical, thermal and/or chemical stimuli, Aδ fibers are the small-diameter myelinated fibers that participate in the transmission of noxious stimuli together with the unmyelinated C-fibers. Myelination problems as well as reduced number of fibers in sciatic nerve have been shown to affect pain perception and pain circuitry function (Chen et al., 2003). While previous findings described alterations in small-caliber axons of animals lacking Tau protein (Harada et al., 1994), and despite the suggestion that Tau protein is implicated in myelination processes (Klein et al., 2002), yet, surprisingly, to the best of our knowledge, there is no in vivo evidence clarifying the role of Tau in nociception. Thus, herein, we assessed pain behavior in animals lacking Tau followed by ultrastructure, molecular and electrophysiological analysis of their sciatic nerve.

Materials and methods

Animals

This study used 4–6 month-old male Tau-null (Tau −/−) and Tau +/+ (C57BL/6) animals (Dawson et al., 2001). Adult Tau-null animals of this strain exhibited no cognitive or locomotor deficits as previously described (Dawson et al., 2010; Gotz et al., 2013). All animals were used in accordance with European Union Council Directive 86/609/EEC and local welfare regulations. Mice were housed in groups of 4 to 5 animals per cage under standard environmental conditions (ambient temperature of 21 ± 1 °C and a relative humidity of 50–60%; 12 h light/dark cycle (lights on at 8:00 A.M.) ad libitum access to food and water).

Pain-related behaviors

Hargreaves' test (acute thermal noxious stimulus)

Animals (N = 18–20 per genotype) were submitted to Hargreaves' test which consists of a radiant heat applied to the plantar skin of the hind limb and withdrawal latency was automatically registered using radiant heat equipment (Plantar Test Device Model 7370, Ugo Basile, Comerio, Italy) as previously described (Pinto-Ribeiro et al., 2013). After acclimatization period, the measurement was repeated 4 times with 60 second intervals. Cut-off time was 10 s. The experimenter was blind to the animals' genotype.

Formalin test (inflammatory stimulus)

The formalin test was performed as previously described (Almeida et al., 1999). Briefly, 50 μL of 5% formalin was injected subcutaneously in the dorsal surface of the right hind-paw. Animals were then placed over a transparent acrylic surface within a 10 cm^3 transparent acrylic box with perforations in all walls for proper air ventilation and sufficient space for animals to freely move. Animals were filmed from below for 60 min and pain-related behaviors (paw protection/licking periods and paw jerks) in the acute (0–5 min) and tonic (15–60 min) phases were posteriorly quantified by two independent observers using EthoLog software (Ottoni, 2000). Prior to the experiment, animals were acclimatized to the experimental conditions to minimize potential stress effects. Experimental groups for Tau-null and Tau +/+ were divided in: saline-injected (control) animals (Sal; N = 10) and formalin-injected animals (Form; N = 13–15). The experimenter was blind to the animals' genotype and drug.

Von Frey test (up-and-down method)

Tactile allodynia (pain evoked by a normally innocuous stimulus) was accessed by the up-and-down method (Chaplan et al., 1994). Animals (10–11 animals per group) were placed in an elevated grid and the glabrous skin of the hind paw was probed with a series of Von Frey calibrated monofilaments: 2.0 g, 1.0 g, 0.6 g, 0.4 g, 0.16 g, 0.07 g, and 0.04 g (North Coast Medical Inc., USA). Starting with the 0.4 g filament, the test would advance upward if no response was elicited (=0) or downward if a brisk withdrawn of the limb was produced (=X) until 6 measurements were obtained around the threshold point according to the model developed by Dixon (1980). The 50% response threshold was then calculated using the formula 50% g threshold = $(10\^[X_f + K.\delta]) / 10.000$, where X_f = value (in log units) of the final Von Frey filament; k = tabular value corresponding to pattern of positive and negative responses (X and O sequences; consult (Chaplan et al., 1994)); δ = mean difference (in log units) between stimuli (0.267). If no response (=O) was obtained up to maximal force (2.0 g) or conversely, if all filaments elicited a response (=X) down to the minimal force (0.04 g), these values were assumed as the 50% withdrawal threshold. The experimenter was blind to the animals' genotype while animals were habituated to the experimental conditions for 2 days before the experiment to minimize potential stress effects.

c-fos immunohistochemistry and density estimation

90 min after formalin test completion, animals were deeply anesthetized with sodium pentobarbital (CEVA Saúde Animal, Portugal) and then perfused through the ascending aorta with PBS followed with 4% paraformaldehyde in PBS. The spinal cord was removed and immersed in the same fixative for 24 h at room temperature (RT) and then, moved to an 8% sucrose solution and kept at 4 °C until use. 50 μm coronal sections of the lumbar area were then obtained in a vibratome (Leica 1000VTS) and serially collected in PBS. For c-fos IHC, sections were incubated in 3.3% H_2O_2 (in PBS, pH 7.2) for 30 min, RT and then, after several washes in PBS and 0.3% PBS-T (Triton-X-100; Sigma), incubated in 2.5% FBS (fetal bovine serum; Stem Cell, 062-00) in PBS-T 2 hour RT, followed by a solution of 1:2000 anti-Fos (Calbiochem, PC38-100) and 2.0% FBS in PBS-T overnight at RT. Sections were then washed several times in PBS-T and incubated for 1 h, RT in a solution of 1:200 biotinylated swine anti-rabbit secondary antibody (Dako, E0353). Sections were then washed in PBS-T and incubated for 1 h, RT in a solution of 1:200 avidin–biotin complex (ABC, Vector Laboratories). Sections were again washed with PBS-T, PBS and Tris–HCL (0.05 M, pH 7.6) and briefly incubated in 0.0005% (w/v) diaminobenzidine tetrahydrochloride (DAB; Sigma Immunochemicals, St. Louis, USA), 0.0002% H_2O_2 (v/v) in Tris–HCl. Sections were then serially placed in Superfrost Plus slides (Menzel-Gläser, Braunschweig, Germany), dehydrated and mounted in Entellan (Merck, Darmstadt, Germany). An Olympus BX51 microscope equipped with U-MAD3 camera was used to acquire images from the left and right dorsal horn of spinal cord. c-fos density was manually determined in specific outer (laminae I–II) and deeper (laminae III–V) regions by an experimenter (AL) blind to the samples provenience (i.e. saline or formalin of both genotypes; 5–6 animals per group).

Sciatic nerve ultrastructure analysis

Sciatic nerves of undisturbed Tau-null and Tau +/+ animals were collected and fixed in 4% glutaraldehyde in 0.1 M sodium cacodylate buffer pH 7.4 for 1 week. Then, they were postfixed in 1% OsO_4, dehydrated and embedded in epon (Electron Microscopy Sciences). 1 μm thick transverse sections covering the complete cross-sectional nerve area were stained with 1% p-phenylene diamine (PPD) for 10 min and mounted on Entellan (Merck). An Olympus DP70 was used to photograph the transverse sections of the nerve and images were mounted on Photoshop. For each animal (N = 5–6 per genotype), the total number of myelinated fibers in one transverse section was counted. The g-ratio [axon diameter / (axon diameter + myelin thickness)] was determined through the analysis of at least 100 fibers. For electron microscopy, ultrathin-sections (60 nm) were counterstained with alcoholic uranyl acetate solution, followed by aqueous uranyl acetate solution and lead citrate. Grids were observed on a JEOL JEM-1400 transmission electron microscope equipped with an Orius Sc1000 digital camera. 16 non-overlapping images were obtained and used for all

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