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Aging, but not tau pathology, impacts olfactory performances and somatostatin systems in THY-Tau22 mice

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ABSTRACT

Somatostatin (SOM) cortical levels decline in Alzheimer's disease (AD) in correlation with cognitive impairment severity, the latter being closely related to the presence of neurofibrillary tangles. Impaired olfaction is another hallmark of AD tightly related to tau pathology in the olfactory pathways. Recent studies showed that SOM modulates olfactory processing, suggesting that alterations in SOM levels participate to olfactory deficits in AD. Herein, we first observed that human olfactory peduncle and cortex are enriched in SOM cells and fibers, in aged postmortem brains. Then, the possible link between SOM alterations and olfactory deficits was evaluated by exploring the impact of age and tau hyperphosphorylation on olfactory SOM networks and behavioral performances in THY-Tau22 mice, a tauopathy transgenic model. Distinct molecular repertoires of SOM peptide and receptors were associated to sensory or cortical olfactory processing structures. Aging mainly affected SOM neurotransmission in piriform and entorhinal cortex in wild-type mice, although olfactory performances decreased. However, no further olfactory impairment was evidenced in THY-Tau22 mice until 12 months although tau pathology early affected olfactory cortical structures. Thus, tau hyperphosphorylation per se has a limited impact on olfactory performances in THY-Tau22 mice.

1. Introduction

Impaired olfaction is a hallmark of normal and pathologic aging, detected in the early stages of Alzheimer's disease (AD) (Mesholam et al., 1998). Sometimes detected before cognitive defects (Djordjevic et al., 2008; Wilson et al., 2009), it has even been proposed as an early marker of AD (Devanand et al., 2010; Olofsson et al., 2009; Wesson et al., 2010b; Westervelt et al., 2008). Indeed, among elderly subjects without cognitive deficits, difficulty in identifying odors predicts subsequent development of mild cognitive impairment (Graves et al., 1999; Wilson et al., 2007). Tau pathology (neurofibrillary tangles or NFTs) is detected early in the olfactory bulb (OB), especially in the anterior olfactory nucleus (AON) (Kovacs, 2004) and correlates to Braak stages (Br.) of neuritic pathology (Attems et al., 2005; Tsuboi et al., 2003): NFTs are found in all definite AD cases (Br. 5/6) (Kovacs et al., 1999; Kovács et al., 2001), in more than 2/3 limbic AD (Br. 3/4), and in more than 1/3 elderly individuals (Br. 1/2) cognitively impaired or not (Attems and Jellinger, 2006). It also affects central olfactory centers (Kovács et al., 2001; Reyes et al., 1993). Tau deposition is highly correlated to OB size reduction in postmortem AD brains (Mundiñano et al., 2011). Clinical dementia correlates with both Braak and olfactory tau scores, indicating that both scores are associated with a high risk of cognitive decline (Attems et al., 2005). By functional magnetic resonance imaging, OB and olfactory nerve atrophy is already measurable in mild cognitive impairment subjects and highly correlated with cognitive deficits (Thomann et al., 2009; Wang et al., 2010). Surprisingly given the recognized link between neocortical tau burden and cognitive impairment severity (Nelson et al., 2012), experimental studies

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directly relating tau hyperphosphorylation status in the olfactory system with olfactory dysfunction are rare in the literature (Cassano et al., 2011; Phillips et al., 2011), as compared with the documented studies of olfactory deficits in APP genetic models (see Guérin et al., 2009; Montgomery et al., 2011; Wesson et al., 2010a, 2011; Wu et al., 2013).

A consistent set of data relates the selective vulnerability of somatostatin (SOM) levels, a major brain inhibitory peptide to normal and pathophysiological aging (reviewed in Epelbaum et al., 2009; Stanley et al., 2012). In AD human cortex or cerebrospinal fluid samples as in experimental models, the decline of SOM levels is correlated with the progression of neuropathologic hallmarks (Ramos et al., 2006; Tan et al., 2010) and the extent of cognitive impairment (Andrews-Zwilling et al., 2010; Bierer et al., 1995; Grouselle et al., 1998; Perez-Cruz et al., 2011). SOM neurons have also been described in human and rodent OB (Bouras et al., 1987; Huang et al., 2013; Lepousez et al., 2010a; Ohm et al., 1988; Saiz-Sanchez et al., 2012) and olfactory cortex (Brunjes et al., 2005, 2011; Saiz-Sanchez et al., 2010, 2014; Suzuki and Bekkers, 2009) together with SOM receptors (SSTR1 to SSTR4, Csaba and Dournaud, 2001; Lepousez et al., 2010a; Martel et al., 2012; Videau et al., 2003). Furthermore, in vivo manipulation of bulbar SSTR2 impacts both OB synaptic activity and olfactory acuity (Lepousez et al., 2010b), demonstrating that endogenous SOM contributes to olfactory processing.

Recently, a significant reduction in SOM interneuron number was reported in the olfactory bulb and cortex of APPxPS1 transgenic mice (Saiz-Sanchez et al., 2012), which develop both olfactory ß-amyloid (Aβ) deposits and olfactory deficits (Rey et al., 2011; Wesson et al., 2010a, 2011). Given the preferential correlation between cognitive impairment severity and neocortical NFTs burden in AD subjects (Nelson et al., 2012) and the major occurrence of NFT in AD olfactory pathways, we took advantage of an experimental murine model to assess the effect of tau pathology on SOM olfactory populations. In the THY-Tau22 strain (Schindowski et al., 2006), hyperphosphorylated tau progressively accumulates in the hippocampus, the amygdala, and other limbic regions together with AD-relevant cognitive changes, in absence of major motor defect or cellular loss (Lo et al., 2013; Schindowski et al., 2006; Van der Jeugd et al., 2011, 2013). Comparing wild-type (WT) and THY-Tau22 mice at 3 crucial stages of the pathology, we characterized hyperphosphorylated paired-helical filament-Tau occurrence in the olfactory pathways and evaluated the impact of aging and tau pathology progression on both olfactory SOM systems and olfactory performances.

2. Methods

2.1. Human samples

Postmortem samples were obtained from brains collected in a Brain Donation Program of the Brain Bank GIE-Neuro-CEB. Autopsy was performed by authorized pathologists after obtaining informed consent in accordance with the French Bioethical laws. Two nondemented control subjects (male, 40 years old, postmortem interval or PMI: 34 hours; female, 91 years old, PMI: 20 hours), 1 AD (female, 89 years old, PMI: 58 hours), and 1 frontotemporal degeneration (FTD) (male, 79 years old, PMI: 44 hours) cases were used in this study. Olfactory bulbs were bilaterally removed from the brain, and olfactory cortex and medial frontal gyrus blocks were dissected out from the left hemisphere (respective Bregma: −1.3 mm and 4 mm, www.thehumanbrain.info).

2.2. Animals

THY-Tau22 mice (C57BL6/J background) were generated by overexpression of human 4-repeat tau mutated at sites G272V and P301S under the control of Thy1.2 promoter (Schindowski et al., 2006). Nontransgenic littermates (WT) were used as controls. Three independent cohorts of age-matched transgenic and WT male mice were used for these studies: experiments were performed at 4, 8, and 12 months for quantitative reverse transcription-coupled polymerase chain reaction (RT-PCR) or behavior and immunohistochemistry studies and at 4, 8, and 15 months for quantitative radioautography. The number of animals included in each experimental group was determined by a power calculation. Standard deviation was assumed from former studies using similar animal model (Lepousez et al., 2010b) to detect a 30% difference. Mice were housed on a 12 hour-light/dark cycle with ad libitum access to food and water (except for olfactory operant conditioning, see the following). All procedures were approved by a local ethics committee (MESR authorization N° 00618.04) in accordance with the European Communities Council Directive (86/609/EU) and performed by observers blind to the genetic status of the mice.

2.3. Immunohistochemistry

2.3.1. Human samples

Autopsic blocks were immediately fixed in 4% paraformaldehyde (PFA) (24 hours for OB and 48 hours for frontal and olfactory cortex samples), cryoprotected in 30% sucrose in phosphate buffered saline for 48 hours and fast frozen at −40 °C in isopentane (Sigma-Aldrich). Sections (50-μm longitudinal [OB] or coronal [cortex]) were performed using a freezing microtome (CM 1325, Leica) and collected in azide-containing phosphate buffered saline (0.05% sodium azide).

For SOM chromogenic staining, sections were washed 3 times in Tris-buffered saline (TBS) and endogenous peroxidase was neutralized with a 15-minute preincubation in 0.3% H_2O_2. Sections were washed and incubated in a blocking solution made of 10% normal donkey serum in 0.3% Triton X100 TBS for 30 minutes. Then, they were incubated for 24 hours in blocking solution with goat anti-somatostatin primary antibody (anti-SOM, # D20, Santa Cruz, 1/400). After 3 TBS washes, sections were incubated for 2 hours with a peroxidase-conjugated donkey anti-goat antibody (Jackson Immunoresearch, 1/500). After 3 TBS washes, revelation was performed with 3,3'-diaminobenzidine (Sigma-Aldrich; 0.5 mg/mL in TBS/0.05% H_2O_2) for 30 minutes. Nissl staining was performed on adjacent sections for each experimental condition.

For fluorescent immunostaining, sections were initially soaked in 25 mM $CuSO_4$ for 1 hour for lipofuscin masking, before the 30-minute normal donkey serum blocking. Then, they were incubated for 24 hour with both goat anti-SOM (1/250) and mouse anti-human phospho-paired-helical filament-Tau (Clone AT8, #MN 1020, ThermoFisher, 1/250) primary antibodies. AT8 antibody is directed against pSer202/pThr205 of human Tau and is routinely used for Braak staging (see Braak et al., 2006). The following day, after 3 TBS washes, sections were incubated for 2 hours with CY3-conjugated donkey anti-goat (Jackson Immunoresearch, 1/1000) and CY5-conjugated donkey anti-mouse (Jackson Immunoresearch, 1/100). After 3 last TBS washes, sections were incubated in thioflavin-S (2.5 mg/mL in 50% EtOH), rinsed, mounted on glass slides, and coverslipped with Fluoromount G (SouthernBiotech).

2.3.2. Mouse samples

Mice (6–9 mice per group) were deeply anesthetized with an intraperitoneal injection of ketamine and xylazine mixture (100 mg/kg/7 mg/kg in saline) and then transcardially perfused with Zamboni fixative (4% PFA, 0.2% picric acid in 0.1 M phosphate buffer pH 7.4). Brains were quickly removed, postfixed 2 hours in 4% PFA, cryoprotected, frozen, and sectioned in 40 μm coronal sections with the same protocol as for human samples. Four consecutive