Peripheral administration of tau aggregates triggers intracerebral tauopathy in transgenic mice

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Soluble tau forms insoluble aggregates following the intracerebral injection of tau inclusions [3, 4]. This is reminiscent of prions, the intracerebral injection of which induces aggregation of cellular prion protein (PrPC) [9]. Multiple routes of administration can transmit prion diseases, with the intracerebral route being more effective than the intraperitoneal route, which is in turn more effective than the oral route [7]. The oral administration of aggregated apolipoprotein A-II [11] and aggregated amyloid protein A (AA) [8] has also been shown to promote systemic amyloidosis. Moreover, intraperitoneally injected aggregated Aβ-containing extracts increased cerebral β-amyloidosis [5]. Here, we report for the first time that the intraperitoneal injection of tau seeds can also induce intracerebral tauopathy.

We used homozygous and heterozygous mice transgenic for human mutant P301S tau [2]. In heterozygous mice, tau aggregation forms later than in homozygous mice, and heterozygous mice live longer than their homozygous counterparts (15 months as opposed to 6 months).

Brainstem extracts from 6-month-old homozygous P301S tau transgenic mice (prepared and analysed as previously described [3]) were injected into the peritoneal cavity of 3-month-old heterozygous mice, an age at which they lack tau deposits. The mice were analysed 9 months after the final injection.

The number of Gallyas silver-positive cells was assessed using a scanning light microscope. Maps of entire brain sections, along with the annotation of the brain outline and artificially altered areas, were used for the counting of black Gallyas-positive and blue haematoxylin-positive structures (Supplementary Fig. 4). Gallyas per cell nucleus (G/N) ratios were counted, thus reflecting the number of silver-positive cells. G/N ratios were calculated per field of view (20× objective, 661.5 × 372.1 μm). Gauss-blurred ratiometric images (sigma = 70 pixels, pixel edge length = 3.45 μm) were superimposed for the control and experimental groups. Subsequently, a pixel-wise Student’s t test was performed and the probability (p) values were plotted, with the statistically significant portion (p < 0.05) being represented as a colour map (Fig. 2). Our ratiometric approach (G/N) only compares equivalent brain regions to each other. Thus, despite anatomical differences in densities of nuclei, our comparative maps remain unbiased.

Although groups of mice injected intraperitoneally (IP) with brainstem homogenate from either P301S tau transgenic mice (with tau filaments) or control (CO) mice (without tau filaments) formed Gallyas-positive structures in the brain, there were also significant differences between the two groups (Fig. 1). G/N ratios (Fig. 2a, left panel) showed a marked increase in the number of silver-positive structures in the brainstem and neocortex of mice IP injected with P301S brainstem extract. The probability map (Fig. 2a, right panel) showed large differences in secondary motor cortex, ventral orbital cortex and olfactory nucleus.
We previously showed that the intracerebral injection of silver-positive brainstem extract from mice homozygous for human mutant P301S tau into the hippocampus and overlying cerebral cortex of 3-month-old heterozygous mice and compared the relative efficiency of intracerebral and intraperitoneal injections after 9 months. Following intracerebral injection, numerous Gallyas-positive structures were present in the CA1 region of the hippocampus and in the secondary motor cortex (Supplementary Fig. 1A). No silver-positive structures were present in these regions following the intraperitoneal injection of control extracts. Heat maps revealed a significant increase in the G/N ratio in the hippocampus following the intracerebral injection of P301S brainstem extract into heterozygous transgenic P301S tau mice (Fig. 2b, left panel). The p maps showed statistically significant differences between groups in the hippocampal area, which was a site of intracerebral injection (Fig. 2b, right panel; Supplementary Fig. 1B). Moreover, the G/N ratio was increased more in brainstem and neocortex following intracerebral than after intraperitoneal injections. Heart, lungs, liver, spleen and kidneys from injected heterozygous P301S tau mice were all silver-negative and tau-negative. No sign of inflammation was detected in either the brain or the periphery.

These findings demonstrate that aggregated tau seeds, such as prions and Aβ aggregates, can reach the central nervous system from the periphery. The underlying mechanisms remain to be determined. The replication of peripherally applied prions and their translocation to the brain are dependent on haematopoietic and stromal immune cells, together with the sympathetic innervation of abdominal lymphoid organs [1]. In mouse models of AA-amyloidosis, peripheral blood monocytes transported the aggregates [10]. It therefore appears that amyloid seeds can be carried by blood cells. This may also be true of aggregated tau. Seeding may require the presence of unfolded monomers [6]. It remains to be determined if tau seeds are able to replicate in the peripheral nervous system. Their transport to the brain may not require endogenous tau, which is not expressed by lymphoreticular cells. This is unlike prion propagation, which depends on the expression of PrPc by stromal and haematopoietic cells. Because the central nervous system is affected in clinical tauopathy, the intracerebral route of seed delivery could be expected to be more effective than the intraperitoneal route. This was the case here, where more silver-positive structures formed in brainstem, hippocampus and neocortex following the intracerebral injection of 5 μl brainstem homogenate (0.19 μg of τ/μl of homogenate) than after the intraperitoneal injection of 200 μl homogenate (0.19 μg of τ/μl of homogenate). It follows that, like prion diseases, tauopathies can be seeded in the brain by tau aggregates delivered peripherally, although the intraperitoneal administration was less effective than the...
intracerebral route. These findings underscore the urgent need for additional work on the aetiology and pathogenesis of tauopathies.

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Fig. 2 Gallyas silver-positive structures per cell nucleus (G/N ratios) in non-injected controls (CO) and in mice injected with aggregated tau-rich homogenate either intraperitoneally (IP) or intracerebrally (IC). a Left panel colour map representing average G/N ratios (CO and IP) of five sagittal brain sections per group. Right panel statistically significant differences were determined by pixel-based t tests and colour coded in the range of <0.05. IP-injected mice had higher G/N ratios in the brainstem and neocortex than CO mice. M2 secondary motor cortex, VO ventral orbital cortex, Olf olfactory nucleus, Pall pallidum, PreOp lateral preoptic area, Hab lateral habenular nucleus, Thal thalamic nucleus, PreTec pretectal nucleus, MesRet mesencephalic reticular formation, Pont pontine reticular nucleus, Gig gigantocellular nucleus, Vest median vestibular nucleus, Sol solitary tract. Dagger symbol an enlarged version is given in Supplementary Fig. 2. Each group consisted of five mice. Asterisk rounding leads to an absolute zero, which reflects an infinitely small value (p is not identical to 0). b Left panel colour map representing average G/N ratios of five sagittal brain sections from IC-injected mice. Right panel statistically significant differences between the three groups were determined by pixel-based t tests and colour coded in the range of <0.05. Statistically significant differences were apparent between non-injected and injected animals, with the major differences between IP- and IC-injected animals being located in the hippocampus, one of the two intracerebral injection sites. Each group consisted of five mice. Asterisk rounding leads to an absolute zero, which reflects an infinitely small value (p is not identical to 0)