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SCIENTIFIC COMMENTARY

Light at the beginning of the tunnel? Investigating early mechanistic changes in Alzheimer's disease

This scientific commentary refers to 'Chemogenetic locus coeruleus activation restores reversal learning in a rat model of Alzheimer's disease', by Rorabaugh *et al.* (doi:10.1093/brain/awx232).

Modern medicine has been increasing life expectancy worldwide. However, longevity comes with a steep price tag; in industrialized countries, more than one-third of those over 85 years of age suffer from dementia, most of them owing to Alzheimer's disease pathology. In this issue of *Brain*, Rorabaugh and co-workers address the lack of suitable experimental models with which to investigate mechanistic changes in early stages of Alzheimer's disease, and add to knowledge about the deleterious effects of locus coeruleus degeneration in the disorder (Rorabaugh *et al.*, 2017).

The hallmarks of Alzheimer's disease pathology include extracellular amyloid-β positive plaques and intraneuronal hyperphosphorylated tau aggregates. These pathological deposits spread following a characteristic topographical sequence, and are directly correlated with, and probably play a role in, neuronal and synaptic loss in affected brain regions. As the majority of neurons are post-mitotic and neurogenesis in the adult human brain is limited, neuronal death marks the 'point of no return' in Alzheimer's disease pathogenesis. Thus, it is reasonable to argue that effective treatment can only be achieved during early phases of the disease when neurons are still viable.

Until recently, Alzheimer's disease was considered a cortical pathology characterized by a retrograde spread of tau pathology from limbic cortex (entorhinal region and hippocampus) through polymodal and primary association cortices to primary sensory cortical fields, a phenomenon captured by the Braak staging system (Braak and Braak, 1991). On the other hand, amyloid-β pathology spreads in an anterograde fashion from polymodal association cortices to allocortex. Involvement of subcortical structures in Alzheimer's disease was deemed to be of secondary importance or occurring only at later disease stages, which indeed seems to be the case for amyloid-β pathology.

About a decade ago, we demonstrated that the serotonergic dorsal raphe nucleus develops Alzheimer-type tau aggregates before the entorhinal cortex does, challenging the concept of the entorhinal cortex as the first brain area to develop Alzheimer-type tau pathology (Grinberg *et al.*, 2009). Around the same time, Braak and colleagues revisited their influential topographical study on the sequence of formation of Alzheimer-type tau aggregates in the human brain (1991), and described how yet another subcortical structure, the noradrenergic locus coeruleus (Fig. 1), also develops Alzheimer-type tau pathology before the entorhinal cortex: now known as Braak stages 0 a–c in their revised staging system (Braak *et al.*, 2011).

Ageing *per se* may affect brain structure and function, to varying degrees, and the threshold between normal ageing and early manifestations of Alzheimer's disease is yet to be defined. In theory, tau aggregates in subcortical nuclei could be harmless without clinical or pathological consequences. Thus, evidence of increasing tau burden and accompanying frank and progressive neurodegeneration are necessary to demonstrate that these subcortical tau aggregates are an integral part of the chain of events associated with early-stage Alzheimer's disease. To clarify the matter, several recent, unbiased, quantitative stereological studies have investigated changes in the locus coeruleus of individuals spanning a broad range of age, cognitive and Braak stages. These studies suggest that accumulation of tau aggregates in the locus coeruleus has deleterious consequences even at early stages. In summary, an average of 8% locus coeruleus neurons show tau aggregates at Braak stage 0, and the tau burden in the locus coeruleus neurons increases linearly with the progression of Braak stages (Ehrenberg *et al.*, 2017; Theofilas *et al.*, 2017). Also, despite the fact that the onset of locus coeruleus

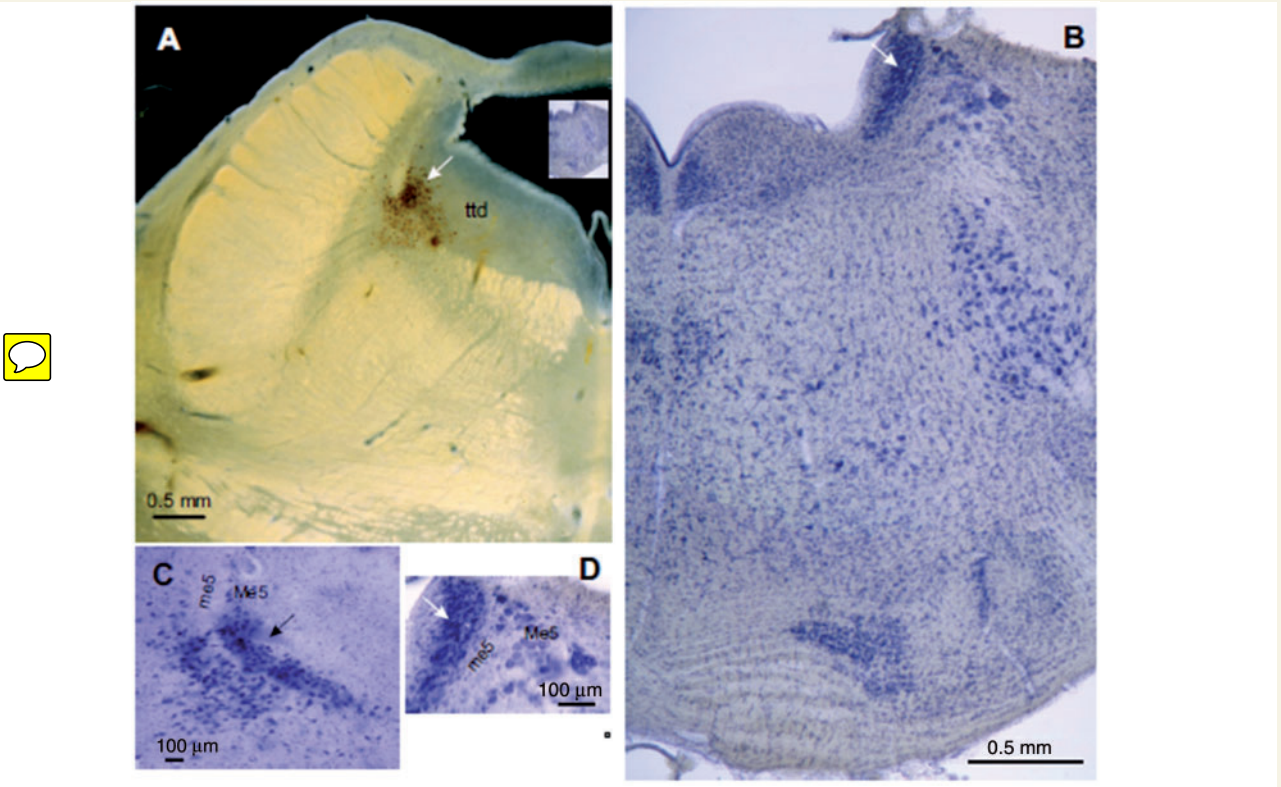

Figure 1 Pontine tegmentum with locus coeruleus in humans and rat. (**A**) Unstained 350-µm thick horizontal section through the left hemi-brainstem of a 78-year-old female with darkfield illumination. Single locus coeruleus cells appear as small light-brown dots due to their rich neuromelanin content. The neurons form a cluster close to the ventromedial border of the trigeminal mesencephalic tract. Neuromelanin is a special feature of the human locus coeruleus, and it originates from metabolized noradrenaline. The functional importance of neuromelanin is a matter of debate. It has been proposed to be a simple waste product indicating high metabolism and interfering with physiological intraneuronal processes particularly in older age, or a buffer of toxic metabolites, or both. (**B**) Gallocyanin (Nissl) stained 110-µm thick coronal section through the right locus coeruleus of a 25-month-old female Han-SPRD rat. To give an idea of the true proportions of human and rat brainstems at nearly equivalent planes of section, an *inset* of **B** was mounted in scale into the fourth ventricle of **A**. Concerning form and internal architecture, human and rat brainstems are quite similar. Myelinated fibre bundles are more numerous in human brainstems, and the outlines of the human fibre bundles show a considerable increase in size. In the floor of the fourth ventricle, a dense fibre plexus consisting of small fibres can be detected in the human brain. The neurons of the medial part of the locus coeruleus are embedded in this fibre plexus, known as the bundle of Schütz or dorsal tegmental tract. The bundle of Schütz carries, most likely, fibres from the central amygdaloid nucleus, hypothalamus and periaqueductal grey and illustrates the intimate relationship of locus coeruleus neurons with these supraspinal nuclei. (**C**) Gallocyanin stained neurons of the right locus coeruleus of a 60-year-old female (section thickness = 400 µm). (**D**) Higher magnification of the locus coeruleus of **B**. Neurons in corresponding tegmental nuclei are bigger in humans, and the density of neurons (average distance between single neurons) is lower compared with rats. This may give a false impression that total locus coeruleus neuronal number is lower in humans than in rats. However, the volume of human brainstem nuclei exceeds that of rats by far. Whereas the total locus coeruleus neuronal number in rats is ~3000–4000, in humans it can go beyond 100 000. The shape of locus coeruleus neurons in humans and rats is similar after Nissl staining and Golgi impregnations, as is their dendritic arborization and likely the efferent noradrenergic connections. Interestingly, neurons of the locus coeruleus, but also the ventral tegmental area, substantia nigra, dorsal raphe and the basal nucleus of Meynert display widespread thin collateralized axons. These neurons are known for their selective disease-specific vulnerability in Alzheimer's disease and Parkinson's disease. In humans, the exponential increase in telencephalic cortical volume, the dense cortical noradrenergic innervation and a relatively low number of noradrenergic locus coeruleus neurons may represent an evolutionary bottleneck (Sharma *et al.*, 2010) that plays an important or decisive role in Alzheimer's disease, a neurodegenerative disorder exclusively manifesting in humans and, perhaps, senile apes. Arrows = locus coeruleus; Me5 = mesencephalic trigeminal nucleus; me5 = mesencephalic trigeminal tract; ttd = dorsal tegmental tract or bundle of Schütz (1891).

neuron loss is protracted compared to the onset of tau aggregation—as is the case in other brain areas—the average neuronal diameter and locus coeruleus volume begin to decrease at Braak stage 1. Locus coeruleus neuronal loss starts at presymptomatic disease stages and correlates with cognitive decline (Arendt *et al.*, 2015; Theofilas *et al.*, 2017). Furthermore, three independent studies using unbiased stereology failed to identify any differences in locus coeruleus volume or neuronal numbers between young individuals and cognitively normal, old-aged controls, all with scarce or absent tau aggregates, suggesting that the structural

locus coeruleus changes identified in successive Braak stages are unlikely to be a result of normal ageing processes (Mouton *et al.*, 1994; Ohm *et al.*, 1997; Theofilas *et al.*, 2017).

Undoubtedly informative, and essential to creating a solid basis for exploring Alzheimer's disease pathophysiology, studies using human post-mortem samples are inherently cross-sectional. As such, they are unsuitable for directly investigating longitudinal changes or for conducting mechanistic assays. Previous cell and animal models for exploring the role of tau biology in Alzheimer's disease have been limited by the lack of *MAPT* (tau gene) mutations resulting in Alzheimer's disease, and by the fact that even transgenic mice with enhanced amyloid-β pathology lack native tau aggregates. Also, studies focusing on the role of locus coeruleus damage in Alzheimer's disease had to resort to approaches causing abrupt locus coeruleus cell loss: an occurrence not observed in humans. Rorabaugh *et al.* now introduce a transgenic rat model for Alzheimer's disease that shows early accumulation of native tau aggregates in the locus coeruleus, overcoming many of the limitations of those previous attempts. As in humans, this rat model (TgF344-AD) overexpressing amyloid-β pathology first shows evidence of intraneuronal, hyperphosphorylated tau aggregates in a subcortical structure, the locus coeruleus. Tau aggregates occur at 6 months of age in these animals, if not before. At this age, the rats show only very scarce amyloid-β deposits. Given that the mean life expectancy of Fisher344 control rats is 30 months, a 6-month period would correspond to 20% of rat life expectancy or a period of 16 years in a human. In these transgenic rats, entorhinal and hippocampal tau aggregates were detected at 16 months old, roughly corresponding to 43 years in humans or more than two decades after the initial appearance of tau aggregates in the locus coeruleus. This is in line with the onset of cognitive decline in humans with autosomal dominant forms of Alzheimer's disease. Next, Rorabaugh *et al.* used this rat model to investigate early locus coeruleus involvement in Alzheimer's disease in the form of tau aggregates in locus coeruleus neurons and their effects on associated brain functions. In absolute numbers, locus coeruleus neuronal counts were similar between transgenic and wild-type rats, even at 16 months of age (locus coeruleus neuronal loss in older rats has yet to be quantified). However, despite the absence of neuronal loss, the transgenic rats showed a significant reduction in noradrenergic innervation of medial entorhinal cortex and hippocampal dentate gyrus at 16 months. Even earlier, at 6 months of age, the transgenic rats showed impaired reversal learning, a hippocampus-dependent task modulated by locus coeruleus noradrenergic innervation. Corroborating these findings, Rorabaugh *et al.* showed that cultured mouse locus coeruleus neurons expressing hyperphosphorylated mutant human tau are viable, but have shorter neurites than controls. The observations in transgenic rats and in mouse locus coeruleus cultures are in line with the findings that locus coeruleus total and neuronal volume begin to shrink when tau starts to aggregate in locus coeruleus neurons, although frank neuronal loss is protracted. This delay between the onset of locus coeruleus changes and locus coeruleus neuronal loss represents a window of opportunity for effective treatment. In fact, Rorabaugh *et al.* were able to reverse the psychometric deficits in TgF344-AD rats by chemogenetic activation of the locus coeruleus.

This study by Rorabaugh *et al.* represents a step forward in understanding the role of precortical accrual of tau aggregates in the locus coeruleus and clearly demonstrates the detrimental effects of Alzheimer-type tau pathology in the locus coeruleus even at early stages. Mounting evidence from patients and experimental models showing the detrimental effects of locus coeruleus degeneration in Alzheimer's disease highlights the need for therapeutic strategies that target locus coeruleus dysfunction, in order to avoid the irreversible functional consequences of damage to these telencephalic projection neurons. Moreover, a number of other brainstem and subcortical nuclei—including the claustrum; select nuclei of the thalamus; ventromedial, tuberomamillary and supra-mammillary nuclei; perifornical and lateral regions of the hypothalamus; peri-peduncular nucleus; substantia nigra and ventral tegmental area; periaqueductal grey; dorsal raphe; and parabrachial nuclei—appear to accrue Alzheimer-type tau aggregates prior to the entorhinal cortex (Stratmann *et al.*, 2016). All of these project to the entorhinal region in a number of mammalian species, including monkeys. Studies in humans and in animal models exploring the biological and clinical consequences of Alzheimer-related degeneration of these nuclei may prove as informative as the studies on the locus coeruleus, and may help to refine understanding of the mechanisms involved in early Alzheimer's disease pathology.

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