Abstract

**Background**: Tauopathies, including Alzheimer’s Disease, are among the most common neurodegenerative diseases in elderly people and cause various cognitive, behavioural and motor defects, but also progressive language disorders. For communication and social interactions, mice produce ultrasonic vocalization (USV) via expiratory airflow through the larynx. We examined USV of Tau.P301L mice, a mouse model for tauopathy expressing human mutant tau protein and developing cognitive, motor and upper airway defects.

**Methodology/Principal Findings**: At age 4–5 months, Tau.P301L mice had normal USV, normal expiratory airflow and no brainstem tauopathy. At age 6–10 months, Tau.P301L mice presented impaired USV, reduced expiratory airflow and severe tauopathy in the periaqueductal gray, Kolliker-Fuse and retroambiguus nuclei. Tauopathy in these nuclei that control upper airway function and vocalization correlates well with the USV impairment of old Tau.P301L mice.

**Conclusions**: In a mouse model for tauopathy, we report for the first time an age-related impairment of USV that correlates with tauopathy in midbrain and brainstem areas controlling vocalization. The vocalization disorder of old Tau.P301L mice could be, at least in part, reminiscent of language disorders of elderly suffering tauopathy.

Introduction

Tauopathies, including Alzheimer’s Disease (AD), are the most prevalent neurodegenerative disorders in elderly people and are characterized by defective learning and memory, besides other cognitive and behavioural symptoms. Tauopathies are accompanied by problems with swallowing, breathing and language. Swallowing disorders, with aspiration of foreign objects often result in pneumonia, a major cause of death in AD [1–3]. Sleep-disordered breathing, with obstructive apnoeas and subsequent hypoxic events possibly contributes to altered brain oxygenation and function in AD [4–8]. Problems with speech and language also develop during AD and in many other neurodegenerative diseases [9–13]. Although clinical and neuroanatomical correlates of progressive language disorders are not well understood, they are often associated with tauopathy-induced alterations of synaptic processes in forebrain networks [10,11,13–16].

Herein we examined vocalization in transgenic Tau.P301L mice, a validated mouse model of tauopathy, produced in the FVB/N genetic background and with specific expression of the human mutant Tau.P301L protein in neurons [17]. From 7–8 months onwards, Tau.P301L mice develop brain tauopathy, cognitive and motor disorders, but also upper airway dysfunction and thereafter breathing defects leading to premature death at 10–12 months [17–20]. For communication and social interactions, mice use ultrasonic vocalization (USV) produced by expiratory airflow through the larynx [21–23]. Different USV patterns, possibly representing different lexicons, behaviours or innate variations in vocal repertoires have been reported in genetically distinct mouse strains [21–26]. We report here for the first time a drastic age-related impairment of USV in transgenic Tau.P301L mice that correlates well with their upper airway dysfunction, reduced expiratory airflow and tauopathy in midbrain and brainstem areas controlling vocalization.


Methods

Ethics Statement

The experiments were performed on adult mice housed with food and water ad libitum, and in accordance with French national legislation (JO 87-848) and European Communities Council Directive (22 September 2010, 2010/63/EU, 74). All animal protocols were approved by our local ethics committee named “Direction Départementale de la Protection des Populations, Préfecture des Bouches du Rhône” (France), with permit numbers A13-505, 13-47 and 13-227 delivered to C. Menuet, Y. Cazals and C. Gestreau, respectively. The procedures for genetic analysis, plethysmography and histology were already reported in detail [17–20].

Animals

Transgenic Tau.P301L mice were produced in the FVB/N genetic background [17,18]. They expressed the longest human tau isoform bearing the P301L mutation (Tau.4R/2N-P301L) under control of the mouse thy1 gene promoter aiming for neuron-specific expression starting in the third postnatal week. We used obligate litters (homozygous Tau.P301L males and females; FVB/N males and females). Transgenic Tau.P301L mice were therefore homozygous for the Tau-P301L transgene and were genotyped by PCR and qPCR. They were compared with age- and sex-matched wild-type FVB/N mice as controls. Mouse rearing environments were similar for both genotypes [21]. Mice were studied at two different ages (different mice at different ages): at 4–5 months because that is pathologically pre-symptomatic and at age 8–10 months, which is in the pathological phase with progressive motor defects, claspings, brain tauopathy, loss of body-weight and breathing defects [17,19].

USV recordings

USV recordings were performed in a custom-build, double-walled concrete acoustic chamber. Conscious, unrestrained mice were placed in clean rectangular polyethylene cages (29×18×12.5 cm) covered by a metal wire lid. A free field microphone (type 4191, Bruel & Kjaer, Denmark) was placed 2 cm above the metal lid, in the centre of the cage. The microphone signal was sampled through audio chip (SoundMax Integrated HD) and dedicated software (Adobe Audition 1.5) at a rate of 192 kHz allowing the recording of the frequency range 10 to 90 kHz, corresponding to the USV range of adult mice [22,27].

The experimental paradigm consisted in placing three mice of the same age and genotype into the USV recording cage. Each group of three mice consisted of one male and two females that had no interactions prior to the recording session, and each group was considered as n = 1 for statistics. Male and female mice were not sexually naïve, female oestrus phase was not checked and no rate of 192 kHz allowing the recording of the frequency range 10

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AT8 and AT100 are monoclonal antibodies specifically directed against phosphorylated human protein tau at epitopes pS198/pT231, and pS202/pS205, respectively (Innogenetics, Gent, Belgium). AT8 is observed in brain of young adults and children and is a marker on the border between physiology and pathology whereas AT100 is a recognized pathological phosphoepitope, typical for AD and primary tauopathies [17,28,29]. AT8 and AT100 expression profiles in brainstem sections were examined using neuroanatomical reference points from mouse atlas [30].

Figure 1. Altered USV production in old Tau.P301L mice. A1 - Rough USV spectrographic display with frequency and time scales in kHz and ms, respectively. A2 – As above but showing the tags (Tg, white dots) placed at slope changes in frequency on the rough USV and the analyzed USV parameters: duration of USV (Dur_{USV}), frequency at tags (Freq_{USV}), max and min Freq_{USV} (RangeFreq), complexity (number of segments delimited by tags), and number of USV produced per min of recording (Nb_{USV}). The total time of USV per min (totT_{USV}) was obtained by summation of individual Dur_{USV}. B – Columns in histograms show Dur_{USV} (expressed in ms) in Tau.P301L (black columns) and FVB/N (white columns) mice at age 4–5 months and 8–10 months (young and old mice, respectively). Note the significant reduction of Dur_{USV} in old Tau.P301L mice. C – As in B but for Nb_{USV} (expressed in USV per min). Note the drastic reduction of Nb_{USV} in old Tau.P301L mice. D – As in B but totT_{USV} (expressed in s per min of recording). Note the significant and drastic reduction of totT_{USV} in old Tau.P301L mice. * indicates a significant inter-strain difference at a given class of age and $ a significant age-related difference for a given strain; ns, non significant inter-strain difference.

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sections were cut, from about 0.5 mm caudal to the pyramidal decussation to about 5 mm more rostral. Sections were stained in groups of three with AT8 (first section), AT100 (second section) or saved (third section). In sections from old Tau.P301L mice, we counted the AT100 positively stained (AT100+) neurons, defined as neurons with visible nucleus, well-marked soma, and high

Figure 2. Altered USV pattern in old Tau.P301L mice. A – Columns in histograms show Freq(USV) (expressed in kHz) used to produce USV in Tau.P301L (black columns) and FVB/N (white columns) mice at age 4–5 and 8–10 months. Note Freq(USV) was similar in old Tau.P301L and FVB/N mice. B – As in A, but for RangeFreq (= max Freq(USV) – min Freq(USV)). Note RangeFreq of old Tau.P301L mice was significantly reduced when compared to that of old FVB/N and that of young Tau.P301L mice. C – Columns in histograms show the distribution of totT(USV) (in %) vs. frequency (Freq), expressed in class of 10 kHz from 30 to 80 kHz in young Tau.P301L and FVB/N at age 4–5 months. Note that most USV used high 50–60 kHz frequency but some used low 30–40 kHz frequency in both genotypes. D – As in C, but for 8–10 months old mice. Arrows highlight that old Tau.P301L mice never used the low 30–40 kHz frequency component in their USV whereas old FVB/N mice still used both low and high frequencies. E – Columns in histograms show the occurrence (%) of USV of different complexity level as defined by the number of segments (see tags in Fig. 1A2) within the USV. Complexity ranged from low (1) to high (5). Note the similar distribution of complexity in Tau.P301L and FVB/N young mice. F – As in E but for old mice. Note the increased occurrence of USV of low complexity and the reduced occurrence of USV of higher complexity (>1) in old Tau.P301L mice compared to old FVB/N and young Tau.P301L mice. Complexity of old FVB/N mice did not change when compared to that of young FVB/N mice. * indicates a significant inter-strain difference at a given class of age and $ a significant age-related difference for a given strain; ns, non significant inter-strain difference.

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staining intensity. Counting neurons twice was not possible because AT100 stained sections are at least 80 μm apart.

Statistics

Statistical analysis of USV and breathing parameters in young and old FVB/N and Tau.P301L mice was performed using ANOVA with post-hoc Newman-Keuls test in the case of normally distributed data, or Kruskal-Wallis with post-hoc Dunn test in the case of non-normally distributed data (Igor Pro software; WaveMetrics, Oregon, USA). Values are given as mean ± standard error of the mean (S.E.M.). Statistical differences were regarded as significant if \( p < 0.05 \).

Results

Ultrasonic Vocalisation (USV) disorders in old Tau.P301L mice

We analyzed USV parameters (Fig. 1A1, A2) of conscious, unrestrained and age-matched Tau.P301L and FVB/N mice. USV parameters were in the same range in young Tau.P301L and FVB/N mice (4–5 months) but differed substantially between old Tau.P301L and FVB/N mice (8–10 months) (Table 1).

In young mice, no significant differences were observed in either the duration of individual USV (Dur(USV); Fig. 1B), or the number of USV produced per min (Nb (USV); Fig. 1C), or the total amount of time spent in producing USV (totT (USV); Fig. 1D). To produce USV, both Tau.P301L and FVB/N young mice used a mean frequency of about 65 kHz (Freq (USV); Fig. 2A). However, Freq(USV) was slightly (6%) but significantly lower in Tau.P301L than FVB/N mice (Table 1). Within the USV, the range of Freq(USV) (RangeFreq = max Freq (USV) – min Freq(USV) ) was similar in Tau.P301L and FVB/N mice (Fig. 2B) as well as the distribution of totT(USV) within the different classes of Freq(USV); (Fig. 2C). Young Tau.P301L and FVB/N mice preferentially used high 50–60 kHz Freq(USV), and occasionally the low 30–40 kHz Freq(USV). We also analyzed the USV complexity by counting the number of segments within USV (tags in Fig. 1A2). In both strains (Fig. 2E), most USV had low complexity (1 or 2 segments).

In the old mice, significant differences were observed, with shorter, more rare and much simpler USV in transgenic Tau.P301L mice than in wild-type FVB/N mice (Table 1). In Tau.P301L mice, the duration of USV decreased with age and Dur(USV) became significantly 2-fold shorter in old relative to young Tau.P301L mice (Fig. 1B). In FVB/N mice, Dur (USV) did not significantly decrease with age, in contrast to the significant 2-fold reduction in old Tau.P301L mice. The number of USV Nb(USV) decreased with age in both strains (Fig. 1C), but the Nb(USV) reduction was dramatic in Tau.P301L mice (20-fold reduction) versus modest in wild-type FVB/N mice (2-fold reduction). Not surprisingly, the substantial reductions of both Dur(USV) and Nb(USV) in young Tau.P301L mice did not significantly decrease with age, in contrast to the significant 2-fold reduction in old Tau.P301L mice. The number of USV Nb(USV) decreased with age in both strains (Fig. 1C), but the Nb(USV) reduction was dramatic in Tau.P301L mice (20-fold reduction) versus modest in wild-type FVB/N mice (2-fold reduction). Not surprisingly, the substantial reductions of both Dur(USV) and Nb(USV) in old Tau.P301L mice dramatically reduced totT(USV) (Fig. 1D); i.e. 30-fold weaker in old than in young Tau.P301L mice. On the other hand, totT(USV) was also significantly reduced in old FVB/N mice but the reduction was modest in FVB/N mice when compared to that of Tau.P301L mice: totT(USV) was 15-fold weaker in old Tau.P301L than in old FVB/N mice. In addition, the USV pattern became simpler and more monotonous in old Tau.P301L than in old FVB/N mice, while the preferential 65 kHz Freq(USV) remained nearly unchanged (Fig. 2A).

Figure 3. Reduced expiratory airflow in old Tau.P301L mice. A - Schematic presentation of the double-chamber plethysmographic set-up allowing the simultaneous recordings of chest spirogram (CSp; in the body chamber) and airflow spirogram (ASp; in the head chamber) in conscious mice. B, C – Averaging of about 100 successive respiratory cycles during quiet period of breathing in young (B) and old (C) mice allowed the measurements of mean ASp and CSp, the calculation of the ASp/CSp ratio and the measurement of expiratory airflow during lung emptying period (gray areas). D - Columns in histograms show the ASp/CSp ratio in Tau.P301L (black columns) and FVB/N (white columns) young and old mice. Note 1) the ratio was similar in young Tau.P301L and FVB/N mice, and 2) the ratio was significantly reduced and increased in oldTau.P301L and FVB/N mice, respectively. E – As in D but for the expiratory airflow. Note the expiratory airflow was similar in young Tau.P301L and FVB/N mice, significantly halved in old Tau.P301L mice and unchanged in old FVB/N mice. * indicates a significant inter-strain difference at a given class of age and $ a significant age-related difference for a given strain; ns, non significant inter-strain difference. doi:10.1371/journal.pone.0025770.g003
40 kHz Freq

Upper Airway dysfunction reduces expiratory airflow in old Tau.P301L mice

USV are produced by expiratory airflow through the larynx [31–34]. From 7–8 months onwards, Tau.P301L mice develop upper airway dysfunction and abnormal expiratory laryngeal activity [19], which may significantly affect their USV.

We therefore measured the expiratory airflow of conscious, young and old transgenic Tau.P301L mice and wild-type FVB/N mice (Table 2). We used double-chamber plethysmography (Fig. 3A) to record the chest spirogram (CSp) produced by the chest respiratory movements in the body chamber and the resulting airflow spirogram (ASp) in the head chamber. We calculated the ASp/CSp ratio, an index of upper airway function [19]. Young Tau.P301L and FVB/N mice had similar ASp/CSp ratio >1, indicative of correct upper airway function (Fig. 3B, 3D).

Measuring the expiratory airflow revealed similar values in young Tau.P301L and FVB/N mice (Fig. 3E). In old mice however, significant differences were observed with reduced ASp and increased CSp in old Tau.P301L mice (Fig. 3C) but not in old FVB/N mice. Moreover, the ASp/CSp ratio was significantly reduced in old Tau.P301L mice (Fig. 3D) but not in old FVB/N mice where it was even significantly increased (Table 2). The ASp/CSp ratio <1 in old Tau.P301L mice corroborated their upper airway dysfunction [19,20]. The expiratory airflow was significantly reduced in old Tau.P301L mice but not in old FVB/N mice (Fig. 3E); it became 2-fold weaker in old Tau.P301L mice relative to young Tau.P301L mice, and to young and old FVB/N mice (Table 2). Despite their upper airway dysfunction, old Tau.P301L mice retained normal respiratory frequency, duration of inspiratory period and duration of expiratory period when compared to the other three groups of mice (Table 2).

Tauopathy develops in brainstem areas of old Tau.P301L mice

From 7–8 months onwards, Tau.P301L mice progressively develop brainstem tauopathy [17,19,20], which may affect central networks controlling upper airways and vocalization. We therefore examined by immunohistochemistry sections of midbrain and brainstem of young and old mice using two distinct antibodies against phosphorylated tau epitopes, AT8 and AT100.

### Table 1. Main USV parameters of young and old Tau.P301L and FVB/N mice.

| Mouse strain | Age    | n   | Dur(USV) | Nb(USV) | totT(USV) | Freq(USV) | RangeFreq |
|--------------|--------|-----|----------|---------|-----------|-----------|-----------|
| Tau.P301L    | Young  | 18  | 51±2     | 132±24  | 6.7±1.1   | 63±2      | 11.2±0.4  |
| Freq(USV)    |        |     |          |         |           |           |           |
| Tau.P301L    | Old    | 18  | 25±2     | 6±0     | 0.2±0.1   | 66±1      | 5.9±0.9   |
| Freq(USV)    |        |     |          |         |           |           |           |
|Tau.P301L old|        |     |          |         |           |           |           |
| FVB/N Young  |        |     |          |         |           |           |           |
| Freq(USV)    |        |     |          |         |           |           |           |
| Tau.P301L    | Old    | 18  | 48±5     | 66±2    | 3.5±1.5   | 64±1      | 9.0±0.7   |
| Freq(USV)    |        |     |          |         |           |           |           |
| Tau.P301L Old|        |     |          |         |           |           |           |

Mean ± SEM values expressed in ms for Dur(USV), number USV per min for Nb(USV), s per min of recording for totT(USV), kHz for Freq(USV) and RangeFreq; n, number of studied mice; p values for inter-strain (Tau.P301L vs. FVB/N) and inter-age (young vs. old) comparisons are considered significant when p<0.05.

### Table 2. Main breathing parameters of young and old Tau.P301L and FVB/N mice.

| Mouse strain | Age    | n   | ASp/CSp | Exp Airflow | Rf   | Ti   | Te   |
|--------------|--------|-----|---------|-------------|------|------|------|
| Tau.P301L    | Young  | 18  | 1.39±0.24 | 1.72±0.11 | 207±9 | 155±14 | 149±11 |
| Freq(NUSV)   |        |     |          |             |      |      |      |
| Tau.P301L    | Old    | 18  | 0.63±0.4 | 0.97±0.09 | 193±10 | 176±13 | 142±7 |

Mean ± SEM values expressed in mL/g/s for expiratory airflow (Exp Airflow), cycle per min for respiratory frequency (Rf), and ms for duration of inspiratory (Ti) and expiratory (Te) periods; n, number of studied mice; p values for inter-strain (Tau.P301L vs. FVB/N) and inter-age (young vs. old) comparisons are considered significant when p<0.05.

40 kHz Freq
AT100, a recognized marker of tauopathy, was not expressed in any of the young or old FVB/N mice (n = 3), and very weakly or practically absent in young Tau.P301L mice (n = 3), while markedly expressed in old Tau.P301L mice (n = 3), as reported previously [17]. The AT100 signal was particularly expressed in midbrain and brainstem sections of old Tau.P301L mice with most dramatic tauopathy in the midbrain periaqueductal gray (PAG), containing the highest density of AT100 positive (AT100+) neurons in the brainstem (Fig. 4). We counted around 100 well-stained AT100+ neurons per PAG section in the three studied old Tau.P301L mice. AT100+ neurons were observed at all rostro-caudal levels of the PAG and in all the PAG sub-regions, i.e. the ventro-lateral part (Fig. 4A2), the dorso-median part (Fig. 4B2) and the dorso-lateral part (Fig. 4B3). Secondly, we observed a high density of AT100+ neurons in the caudal retroambiguus (NRA) in the caudal medulla (Fig. 5A) and in the Kolliker-Fuse (KF) nucleus in the dorso-lateral pons (Fig. 5B). AT100+ neurons delimited the KF area, extending between the middle and superior cerebellar peduncles, below the lateral parabrachial nucleus and above the principal sensory trigeminal nucleus (Fig. 5B). We counted around 25–50 well-stained AT100+ neurons per sections all along the whole rostro-caudal extension of the KF (about 700–1000 μm). In the caudal medulla, frequent AT100+ neurons were also found in the NRA area, previously defined in mouse brainstem [35]. We consistently counted around 10 well-stained, packed AT100+ neurons per studied section from about 500 μm caudal to 500 μm rostral to the pyramidal decussation. Aside the PAG, KF and NRA areas, AT100+ neurons were observed scattered in the whole brainstem, but were especially dense in the raphe obscurus, raphe magnus, locus coeruleus (data not shown), oral pontine reticular nucleus and subcoeruleus (Fig. 5B).

Conversely, some other areas appeared markedly spared by tauopathy. We found almost no AT100+ neurons in the nucleus ambiguus (nA) and the nucleus tractus solitarius (nTS) (Fig. 5C).

**A – Caudal periaqueductal gray area**

**B – Rostral periaqueductal gray area**

*Figure 4. Tauopathy in the PAG of old Tau.P301L mice.* Immunohistochemistry with AT100 as tauopathy marker on midbrain coronal sections of old Tau.P301L mice reveals dramatic tauopathy in the whole PAG, affecting both its caudal (A) and rostral (B) parts. A2, B2 and B3 are enlargements of the dotted line boxes drawn in A1 and B1, and show high density of AT100+ neurons in the caudal, ventro-lateral PAG (A2), the rostral, dorso-median PAG (B2) and the rostral dorso-lateral PAG (B3) of the same old Tau.P301L mouse. B4 shows frequent AT100+ neurons in the rostral, dorso-lateral PAG of another old Tau.P301L mice. Calibration bars: 500 μm for A1, B1; 100 μm for A2, B2-B4.

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the raphé dorsalis (Fig. 5B) and most of the cranial motor nuclei (data not shown). The hypoglossal motor nucleus was also generally spared by tauopathy but some AT100+ neurons were observed in the caudal part of the hypoglossal nucleus of one of the three studied Tau.P301L mice (Fig. 5A). In addition, as previously reported in Tau.P301L mice at the terminal stages [20], the respiratory-related areas implicated in respiratory rhythmogenesis (in the ventral medulla below the nA) and in central chemosensitivity (in the very ventral medulla below the facial motor nucleus) did not contain significant amount of AT100+ neurons.
The epitope defined by AT8 is a marker on the border between physiology and pathology, and we confirmed its previously reported expression profile in the brainstem of old Tau.P301L mice [19,20]. Dense AT8 expression was evident in the PAG, KF and NRA areas, and more scattered in the whole reticular formation, with increased density in the raphe obscurus and magnus, locus coeruleus (data not shown). Only rare AT8+ neurons were in the nA, the nTS, the hypoglossal motor nucleus (some in one mouse), the other motor cranial nuclei and the respiratory-related areas of the ventral medulla (data not shown).

To summarize, both AT100 and AT8 revealed severe tauopathy in old Tau.P301L mice, especially affecting the midbrain PAG, pontine KF and medullary NRA areas, known to control upper airway and vocalization.

**Discussion**

Compelling evidence exists that mice produce USV for communications and social interactions [21–23] and that mouse models of Angelman Syndrome [24], autism [25] or AD [26] produce specific USV. However, these aspects have been studied mainly in young dams and pups, not in aging mice. In the aging Tau.P301L model of tauopathy [17,18], we report for the first time a dramatic age-related impairment of USV which may be, at least in part, reminiscent of progressive language disorders of elderly people suffering tauopathy and neurodegenerative diseases.

**USV impairment of Tau.P301L mice is linked to upper airway dysfunction**

Respiration and vocalization are two tightly linked motor acts that implicate the same groups of chest, abdominal and upper airway muscles. USV emission originates from expiratory airflow through the larynx as demonstrated by larynx excision, tracheotomy and motor nerve transection [31–34]. The motor neurons controlling the laryngeal muscles belong to the nA, with intermingled dilatator and constrictor motor neurons. They are multi-functional neurons driven by several central pattern generators, including those for respiration, vocalization and swallowing [36]. During USV, the inspiratory dilatator nA neurons become silent meanwhile the expiratory constrictor nA neurons are activated, mostly prior to USV [37,38].

From 7–8 months onwards, Tau.P301L mice develop upper airway dysfunction [19]: an inspiratory shift of the period of activity of expiratory laryngeal motor neurons induces a paradoxical tendency to laryngeal closure during inspiration, which subsequently reduces air entry within the lungs. Consistent with these previous results, we report here that the expiratory airflow is halved in old Tau.P301L mice. Thus the abnormal laryngeal motor activity and the expiratory airflow reduction highly likely contribute to reduce the ability of old Tau.P301L mice to produce frequent, long-lasting and complex USV. Conversely, old wild-type FVB/N mice retain normal expiratory laryngeal discharge [19], normal expiratory airflow and rather spared USV, with only a modest reduction of Nb(USV) and toT(USV). As discussed below with AT100 expression, the impairments of laryngeal discharge, expiratory airflow and USV production in old Tau.P301L mice do not originate from a direct alteration of laryngeal motor neurons but result from alteration of their central drivers.

**USV impairment of Tau.P301L mice originates from PAG, KF and NRA tauopathy**

We previously reported that Tau.P301L mice develop brainstem tauopathy from 7–8 months onwards, with frequent AT8+ neurons in the KF revealing an altered control of upper airway function [17,19]. Here, we used the late tau pathological marker AT100 to confirm the KF alteration in old Tau.P301L mice and reported numerous AT100+ neurons in the pontine KF but also the midbrain PAG and medullary NRA nuclei. These three structures are crucial for the control of upper airway function and vocalization as illustrated in the summary diagram of Fig. 6. The PAG is the main descending relay of the emotional motor system which converts higher emotional and cognitive commands into motor activity for complex behaviours, including respiration, vocalization and copulation [39–42]. The PAG projects to both the KF [43] and the NRA [40,42,44]. It also targets many other structures such as the locus coeruleus [45,46], the rostro...
ventromedial medulla [47] and the raphé magnus [48,49]. The PAG does not directly control the chest, abdominal and upper airway motor neurons but uses the NRA as a relay [50] and the NRA in turn projects to the PAG [51]. The NRA contains multifunctional pre-motorneurons that target the laryngeal nA motor neurons and the thoraco-abdominal motor neurons [35,44,50,52,53] and controls their activity during respiration [54], vocalization [42,50,53], coughing, sneezing [56,57] and copulation [42,58]. The KF modulates the activity of laryngeal and tongue motor neurons [59–61], controlling the upper airway function [19], the expression of learned upper airway behaviours [62] and the vocal patterning [63].

We propose that tauopathy-induced alterations of the crucial PAG, KF and NRA networks play a major role in the USV impairment of old Tau.P301L mice, while not excluding the implication of additional networks. Currently, no data are available in the literature on olfaction, hearing and vision of 8–10 months old Tau.P301L mice and we have observed no obvious defects in sniffing and hearing that could explain their USV impairments. Old Tau.P301L mice develop tauopathy in cortical and thalamic areas [17], which may impact on their social interactions, emotional status and subsequently USV, possibly via the PAG, KF and NRA relays. Old Tau.P301L mice also develop laryngitis in the locus coeruleus and some raphé nuclei, which may affect the monoaminergic modulations. USV are affected by the serotoninergic system [64] and the serotonin metabolism of Tau.P301L mice becomes abnormal at terminal stages of the disease [20]. On the other hand, AT100+ neurons are rare, almost absent in the nA, which excludes a direct alteration of nA motor neurons and reinforces the concept of indirect effects via alterations of PAG, KF and NRA networks. Similarly, AT100+ neurons are rare in the nTS where the peripheral respiratory inputs are integrated and in the ventral medulla where the respiratory rhythm is, at least in part, generated. Consistently, old Tau.P301L retain normal respiratory frequency, duration of inspiration and duration of expiration (present results) and developed marked breathing defects only at terminal stages of the disease [20].

Translational aspects of mouse USV impairment to progressive language disorders

In mice, the different strain-specific USV patterns are viewed as different lexicons or innate variations in vocal repertoires [21–26]. USV are proposed as models for speech and socio-cognitive disorders [65] and for drugs and genes effects on social motivation, affect regulation and communication [21]. It is outside the scope of this study to define or speculate on the USV impairment in old Tau.P301L mice in terms of social interactions, lexicon or semantic defects. However, the possible link between the USV impairment in Tau.P301L mouse and the progressive language disorders in patients is worth noticing.

Aging from 4–5 to 8–10 months had only minor effects on USV of wild-type FVB/N mice: it significantly but modestly reduced Nb(USV) and totT(USV), and had no significant effect on Dur(USV). RangeFreq, use of low Freq(USV) components and complexity. In contrast, aging had major effects on USV of Tau.P301L mice where tauopathy not only exacerbated the modest age-related reduction of USV observed in FVB/N mice, dramatically reducing the quantitative USV parameters (Dur(USV), Nb(USV) and totT(USV)), but also significantly altered the qualitative USV parameters (RangeFreq, use of low Freq(USV) components and complexity). Indeed, analyzing Tau.P301L at intermediate ages between 5 and 8 months might be highly informative about the link between histopathology and functional USV deficits.

In healthy humans, normal aging affects respiration and vocalization, reducing the ability to generate the required air pressure for speech production [66,67]. Old persons initiate speech at a higher lung volume and produce fewer syllables per breath than young adults [68,69]. These reductions of speech performance during normal aging are negligible compared to pathological language disorders occurring with tauopathy. Progressive language disorders concern a group of clinically, genetically and pathologically heterogeneous neurodegenerative disorders, with different variants based on motor speech, linguistic and cognitive features [9,15,70,71]. However, neither language disorder phenotyping nor brain imaging alone appears a reliable predictor of pathology [72]. A few case reports suggest possible links between language disorder, swallowing impairment, respiratory difficulties and brainstem alterations [73–77]. In addition, PAG abnormalities have been reported in some cases of mutism [78], in AD [79] and possibly in frontotemporal dementia [80] and Parkinson disease [81]. But these reports are rare and atypical when compared to the plethora of reports about forebrain imaging and language disorders.

In a mouse model for tauopathy, we report an age-related impairment of vocalization accompanied with tauopathy of the PAG, KF and NRA network controlling vocalization. As it cannot be excluded that the PAG, KF and NRA network is also altered in elderly suffering tauopathy [73–81], we suggest that imaging studies in old patients with progressive language disorders concern not only the forebrain but also the midbrain and brainstem structures.

**Author Contributions**

Conceived and designed the experiments: CM YC. Performed the experiments: CM YC. Analyzed the data: CM. Contributed reagents/materials/analysis tools: YC. Wrote the paper: GH FVL CG MD. Designed the software used for ultrasonic vocalization analysis: YC. Performed the immunohistological work: PB LG.

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