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# Intraneuronal accumulation of C99 contributes to synaptic alterations, apathy-like behavior, and spatial learning deficits in 3×TgAD and 2×TgAD mice

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ABSTRACT

The triple transgenic mouse model (3×TgAD: APPswe, $Tau_{P301L}$, $PS1_{M146V}$) recapitulates both amyloid β (Aβ)- and tau-related lesions as well as synaptic and memory deficits. In these mice, we reported an early apathy-like behavior and alterations in synaptic plasticity appearing concomitantly with intraneuronal accumulation of C99 in the subiculum. To delineate the genuine contribution of C99 on the above phenotypes, we generated double transgenic mice (2×TgAD: APPswe, $Tau_{P301L}$) that accumulate C99 without Aβ deposition or hyperphosphorylation of tau and compared them to 3×TgAD mice. Here, we show that both TgAD mice display similar decreases in long-term potentiation and in spontaneous locomotor activity measured by actimetry suggesting that the synaptic alterations and the apathy-like behavior were likely linked to C99 rather than Aβ. However, spatial learning alterations, assessed by the Morris water maze task, are more pronounced in 3×TgAD than in 2×TgAD, suggesting that both Aβ and C99 contribute to defects in the acquisition of spatial information. Finally, even if similar results are observed in males, cognitive and non-cognitive deficits are more severe in females.



## 1. Introduction

Alzheimer's disease (AD) is an age-related disorder responsible for the most common form of dementia in the world. Histologically, it is characterized by two abnormal protein deposits: extracellular senile plaques mainly composed of hydrophobic amyloid β (Aβ) peptides and neurofibrillary tangles made of intracellular filamentous aggregates of the hyperphosphorylated tau protein. One of the etiological hypotheses concerns the amyloid cascade that proposes a key role of Aβ in AD setup and progression (Hardy and Higgins, 1992; Selkoe and Hardy, 2016). Aβ peptides are produced from their precursor, the β-amyloid precursor protein (βAPP) in the amyloidogenic pathway. βAPP is first cleaved by the β-secretase to release a C-terminal fragment, C99, which then undergoes subsequent hydrolysis by the γ-secretase to yield not only Aβ but also the cytosolic APP Intracellular Domain (AICD) that controls the transcription of several genes related to AD (Pardossi-Piquard and Checler, 2012). An alternative non-amyloidogenic pathway by α-secretase generates another C-terminal fragment, C83. This cleavage precludes Aβ production but does not impair γ-secretase–mediated production of AICD.

Because senile plaques are poorly correlated with the severity of the disease, it has been suggested that the early occurrence of intracellular Aβ and soluble Aβ oligomers rather than senile plaques could be responsible for the first pathological events (LaFerla et al., 2007; Larson and Lesne, 2012; Wirths et al., 2004). However, several lines of evidence suggest that some Aβ-independent mechanisms could also contribute to AD-related anatomical and functional alterations (Pimplikar et al., 2010).

We have recently reported that the intraneuronal aggregation of C99 induces an Aβ-independent lysosomal-autophagic dysfunction, in vivo, in triple transgenic mice (3×TgAD). Interestingly, this phenotype can be mimicked by the viral expression of C99 in wild-type mice (AAV-C99 mice), and it is exacerbated by the pharmacological blockade of γ-secretase (Lauritzen et al., 2016). These findings agreed with previous studies showing that C99 accumulation induces axonal transport impairment and morphological and

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functional abnormalities of early endosomes (Jiang et al., 2010; Kim et al., 2016). Although these studies support the idea that C99, independently to Aβ, could contribute to molecular and cellular dysfunctions taking place in AD pathology, little is known concerning the contribution of C99 on cognitive and behavioral deficits observed in AD mouse models.

The 3×TgAD mouse model first generated and described by LaFerla is broadly used in AD field. These mice expressing mutated amyloid precursor protein and tau proteins ($APP_{swe}$ and $Tau_{P301L}$), and physiological levels of mutated PS1 protein ($PS1_{M146V}$), develop both amyloid plaques and neurofibrillary tangles (Oddo et al., 2003b). Synaptic dysfunction and cognitive deficits occur before plaque or tangle formation and could be temporally correlated with intraneuronal Aβ-related immunoreactivity rather than with extracellular Aβ deposits (Billings et al., 2005). However, by both genetic and pharmacological approaches, we have demonstrated that this intracellular staining did not correspond to Aβ itself but rather to its direct precursor C99 (Lauritzen et al., 2012). Moreover, we found that this early C99 accumulation was both spatially and temporally associated with the first synaptic alterations impacting long-term potentiation (LTP) (Pardossi-Piquard et al., 2016). Interestingly, 3×TgAD mice displayed an early and drastic decrease of spontaneous activity measured by actimetry that could be assimilated to an apathy-like phenotype (Pardossi-Piquard et al., 2016). This neuropsychiatric symptom is the most frequent and earliest non-cognitive behavior observed in AD patients (Zhao et al., 2016) and in clinical trials, apathy is associated with a decreased activity that can be monitored by actigraphy (David et al., 2012; Konig et al., 2014).

By crossing 3×TgAD and non transgenic wild-type (WT) mice, we developed another AD mice model, the double transgenic mice (2×TgAD) harboring both $APP_{swe}$ and $Tau_{P301L}$ mutant proteins but expressing endogenous wild-type PS1. Similarly to 3×TgAD mice, 2×TgAD mice exhibit early accumulation of C99 in the subiculum. However, in contrast to 3×TgAD mice, 2×TgAD mice display undetectable Aβ and fail to produce extracellular plaques, even at very late ages of the pathology (Lauritzen et al., 2012). Moreover, hyperphosphorylation of tau is not observed in 2×TgAD mice even at 20 months of age. Thus, the comparison between 3×TgAD and 2×TgAD mice seems particularly adequate to investigate the contribution of C99 to synaptic and behavioral alterations independently of Aβ and tau pathology. Here, we report that 3×TgAD and 2×TgAD mice have similar decreases in spontaneous activity and LTP suggesting that apathy-like behavior and synaptic alterations are not associated with Aβ but more likely with C99. In contrast, learning impairment assessed by the Morris water maze (MWM) task is stronger in 3×TgAD compared to 2×TgAD mice indicating that besides C99, Aβ could also contribute to deficient acquisition of spatial information. Finally, our study did not unravel major sex-related differences, even if we observed more pronounced phenotypes in females.

## 2. Materials and methods

### 2.1. Animals

3×TgAD ($APP_{swe}$, $Tau_{P301L}$, and $PS1_{M146V}$) and nontransgenic (WT) mice colonies were maintained from breeding pairs generously provided by Dr LaFerla (Oddo et al., 2003b). As described previously, 2×TgAD expressing $\beta APP_{swe}$, $Tau_{P301L}$, and wild-type PS1 were obtained in our laboratory by crossing the 3×TgAD with these WT mice (Lauritzen et al., 2012). Therefore, the same WT mice with the original hybrid background (129-C57BL/6) were used as control for both 2×TgAD and 3×TgAD mice. Each strain line is maintained by intercross breeding and backcrossed with the original strain every 10 generations. Males and females were housed with a 12:12 hour light/dark cycle and were given free access to food and water. All experimental procedures were in accordance with the European Communities Council Directive of 22 September 2010 (2010/63/EU) and approved by the French Ministry of Higher Education and Research (project number #00253.02).

### 2.2. Actimetry

Spontaneous locomotor activity was measured using an actimeter apparatus (Actimeter system, Imetronic, France), as previously described (Pardossi-Piquard et al., 2016). Briefly, the cages were illuminated 12 hours per day starting from 8 AM. Each cage was equipped with infrared captors at each side of the box and connected to an electronic interface that allowed the automatic measurement of spontaneous locomotor activity (rearing, front activity, back activity, and locomotion). Phenotypes obtained in 12-month-old WT (28 females and 16 males), 2×TgAD (2AD, 24 females and 16 males), and 3×TgAD (3AD, 22 females and 16 males) mice were statistically analyzed by 2-way analysis of variance (ANOVA) (genotype × sex) applied with Benjamini-Hochberg correction using InVivoStat statistical software.

### 2.3. Morris water maze

At least 2 weeks after actimetry analysis, a group of 12-month-old WT (16 females and 16 males), 2×TgAD (2AD, 13 females and 15 males), and 3×TgAD (3AD, 16 females and 16 males) mice was assessed in the MWM task conducted in a circular pool 90 cm in diameter filled with water made opaque with nontoxic white dye opacifier (Viewpoint). Pool temperature was maintained at 24 °C. The maze was located in a room containing several distal extramaze posters helping mice to orientate. The circular escape platform, 10 cm in diameter, was submerged in the center of 1 quadrant of the pool and remained in the same position throughout the learning trials. During 5 days, each mouse performed 4 trials per day. For each trial, the mouse was placed into the pool at one of 3 different designed start positions located in all quadrants not housing the platform. Mice were allowed to find the platform during 60 seconds. If the mouse found the platform before 60 seconds, it was left 20 seconds on the platform then removed from the pool. If the platform was not found after 60 seconds of swimming, the mouse was manually guided to the platform and allowed to reorient to the distal visual cues for an additional 30 seconds before being removed from the pool. For each day, the latency to find the platform was averaged and statistically analyzed by mixed model ANOVA (genotype × sex × training day) using InVivoStat statistical software. The means of mice swim speed were calculated for each group during the last trial and analyzed by 1-way ANOVA using InVivoStat statistical software. The visual cued test was performed in females after the hidden platform training. During the visual cued test, each mouse performed 3 consecutive trials in the pool filled with opaque water. The mouse was given 60 seconds to find the platform placed above the water level and made visible using a flag mounted above the platform. For each trial, the mouse was placed at the same start position, and the visible platform was moved to different locations between each trial. Latency to find the visible platform was averaged for 12-month-old WT (16 females), 2×TgAD (2AD, 13 females), and 3×TgAD (3AD, 16 females) mice and analyzed by 1-way ANOVA using InVivoStat statistical software.

### 2.4. Rotarod test

Before the MWM task, motor performance was measured using a Rotarod apparatus (Bioseb, model LE8200). Mouse was placed on the rotating rod, and the latency to fall was measured while the