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Detection of all adult Tau isoforms in a 3D culture model of iPSC-derived neurons

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ABSTRACT

Tauopathies are a class of neurodegenerative diseases characterized by the presence of pathological intracellular deposits of Tau proteins. Six isoforms of Tau are expressed in the adult human brain, resulting from alternative splicing of the *MAPT* gene. Tau splicing is developmentally regulated such that only the smallest Tau isoform is expressed in fetal brain, contrary to the adult brain showing the expression of all 6 isoforms. Induced Pluripotent Stem Cell (iPSC) technology has opened up new perspectives in human disease modeling, including tauopathies. However, a major challenge to *in vitro* recapitulation of Tau pathology in iPSC-derived neurons is their relative immaturity. In this study, we examined the switch in Tau splicing from fetal-only to all adult Tau isoforms during the differentiation of iPSC-derived neurons in a new 3D culture system. First, we showed that iPSC-induced neurons inside Matrigel-coated alginate capsules were able to differentiate into cortical neurons. Then, using a new assay that allowed both the qualitative and the quantitative analysis of all adult *MAPT* mRNA isoforms individually, we demonstrated that BrainPhys-maintained neurons expressed the 6 adult *MAPT* mRNA transcripts from 25 weeks of maturation, making this model highly suitable for modeling Tau pathology and therapeutic purposes.

1. Introduction

Tau is a multifunctional protein, originally identified as a cytoplasmic protein associated with microtubules. Besides its role in tubulin assembly and microtubule stabilization, recent reports have revealed several novel and atypical roles of Tau, such as regulation of neuronal activity, synaptic plasticity, DNA/RNA protection, maintenance of the integrity of genomic DNA and stability of pericentromeric heterochromatin (Sotiropoulos et al., 2017).

The human Tau protein is encoded by a single gene, *MAPT*, which maps to chromosome 17q21.31. Alternative mRNA splicing of exons 2, 3 and 10 generates 6 major adult brain-specific isoforms ranging from 352 to 441 amino acids in length. These isoforms differ with regard to the number of inserts of 29 amino acids (encoded by exons 2 and 3), and the inclusion, or not, of the 31 amino acids microtubule-binding

repeat encoded by exon 10. Isoforms containing 0, 1 or 2 amino-terminal inserts are known as 0N, 1N or 2N, respectively. The isoforms are also categorized depending on whether they contain 3 (3R) or 4 (4R) carboxyl-terminal repeats. In the developing human brain, only the shortest Tau isoform 0N3R is present (Goedert et al., 1989). The Tau expression pattern is modified post-developmentally with a reduction in 0N3R levels and the expression of all 6 isoforms. In the adult human brain, 3R and 4R isoforms are found at an equimolar ratio. The 2N species are underrepresented in comparison to 0N and 1N isoforms, such that 2N, 1N and 0N isoforms make up 9%, 54%, and 37% of the total, respectively (Goedert and Jakes, 1990; Hong et al., 1998).

The accumulation of pathological intracellular deposits of the Tau protein is the hallmark of numerous neurodegenerative disorders referred to as tauopathies (Sergeant et al., 2008). Importantly, isoform composition, morphology and anatomical distribution of cellular Tau-

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deposits are defining distinct tauopathies. In Alzheimer's disease (AD), all 6 Tau isoforms are abnormally phosphorylated and aggregated. In Pick's disease, 3R-Tau predominates, whereas 4R-Tau aggregation is found in corticobasal degeneration and progressive supranuclear palsy. In addition, > 50 deleterious *MAPT* mutations have been identified in familial frontotemporal dementia and parkinsonism linked to chromosome 17 (FTDP-17). Known mutations produce either a reduced ability of Tau to interact with microtubules or other partners, or an overproduction of 4R-Tau isoforms, resulting in a change in the ratio of 3R to 4R isoforms.

During the past decade, human induced Pluripotent Stem Cell (iPSC) technology has opened up new perspectives in human disease modeling. Several groups have already demonstrated that iPSC-derived neurons may be a valuable tool to study tauopathies. However, a major challenge to *in vitro* recapitulation of Tau pathology is the relative immaturity of the iPSC-derived neurons. Numerous studies showed that wild-type iPSC-derived cortical neurons express predominantly the ON3R embryonic Tau isoform (Biswas et al., 2016; Hallmann et al., 2017; Imamura et al., 2016; Iovino et al., 2015; Sato et al., 2018; Silva et al., 2016; Verheyen et al., 2018; Verheyen et al., 2015). Even if most of these studies described a switch in Tau splicing from only 3R isoforms to both 3R and 4R isoforms during the differentiation procedure, the shortest Tau ON3R isoform remained overwhelmingly majority. Extended culturing time up to 365 days allowed the detection of ON3R, ON4R, 1N3R and 1N4R-Tau protein isoforms in iPSC-derived neurons (Iovino et al., 2015; Sposito et al., 2015), but still with a predominance of ON3R. The presence of adult Tau isoforms has also been described at RNA levels in iPSC-derived cortical neurons (Ehrlich et al., 2015). However, the corresponding protein isoforms were not detected. Only the fetal Tau isoform ON3R was present in these iPSC-derived cortical neurons. Therefore, optimization of differentiation protocols to accelerate the maturation of neurons and the acquisition of mature Tau splice variants remain a challenge to study tauopathies, notably the Tau 4R-related tauopathies, in this model.

It has been shown that a 3D microenvironment enhanced the maturation of iPSC-derived neurons and was associated with an increase of 4R isoform expression (Choi et al., 2014). A new neuronal 3D culture system, based on the use of Matrigel-coated capsules, was recently described (Alessandri et al., 2016). This cell culture container allows the differentiation of encapsulated neuronal stem cells into neurons *in situ* and long-term culture. In this study, we examined iPSC-induced neuronal cell maturation and the switch in Tau splicing from fetal-only to adult Tau isoforms during the differentiation procedure in this novel 3D microenvironment. Importantly, all *MAPT* mRNA isoforms were individually analyzed and accurately quantified using a new assay based on the simultaneous amplification of the 6 isoforms. We tested two cell culture media: a commonly used medium (DMEM/F12 – Neurobasal) and the BrainPhys neuronal medium that was designed to increase the proportion of synaptically active neurons.

2. Material and methods

2.1. iPSC line and encapsulation

BC-1 (WT XY, passages 15–25, MTI-Globalstem, Gaithersburg, MD) iPSC line was maintained under feeder free conditions. Culture plates were coated with Matrigel matrix 2 h at 37 °C (Corning, NY, 1/100, diluted in DMEM medium.). BC-1 colonies were routinely sub-cultured using ReLeSR (STEMCELL Technologies, Vancouver, Canada). iPSC were cultured in mTESR1 (STEMCELL technologies) complemented with 1% penicillin/streptomycin (Invitrogen, Carlsbad, CA). Cultures were fed daily and passaged every 5 to 7 days.

2.2. Encapsulation procedure

The encapsulation process is described in detail in (Alessandri et al.,

2016). Briefly, it consists in using a coextrusion microfluidic device that has been microfabricated using a 3D printer (MicroPlus, Envisiontec). This device is composed of three coaxial capillaries, which are loaded with three different solutions through direct connection to syringes mounted to high precision low pressure pumps (neMESYS, Cetoni). A solution of sodium alginate (Protanal LF200S; FMC) at 2% wt/vol in water flows in the outermost channel. The intermediate capillary is filled with a 300 mM sorbitol (Merck) solution. The innermost phase is a suspension of iPSC at 2×10^6 cells per mL in sorbitol solution and mixed with Matrigel at 25% vol/vol. The respective flow rates selected for the present work were respectively 20/20/30 mL.h⁻¹ for the inner/intermediate/outer solutions. The compound liquid droplets are generated at the outlet of the nozzle (of 130 µm in diameter) at a high rate (> 103 per sec) and fall into a gelation calcium bath (calcium chloride 100 mM). Cells are thus entrapped into hollow alginate hydrogel shells (radius ~150 µm) that are subsequently transferred (within < 1 min) in the culture medium. As described in (Alessandri et al., 2016), great care was taken to keep Matrigel below room temperature during the encapsulation process in order to avoid Matrigel gelation prior to capsule formation. Matrigel was found necessary to promote the survival and growth of BC-1 inside the capsules.

2.3. Generation of cortical mature neurons

Neural induction was carried out in 1:1 mix of DMEM/F12 and Neurobasal supplemented with B27 and N2 (Thermo Fischer Scientific Inc., Waltham, MA), 1 µM LDN-193189 (Sigma Aldrich, St-Louis, MO) and 10 µM SB431542 (Tocris Biosciences, Bristol, UK). Medium was changed every day during 8 days. Then, differentiation of Neural Stem Cells was achieved using the DMEM/F12:Neurobasal mix (1:1) supplemented with B27 and N2, or the BrainPhys medium supplemented with N2-A and SM1 (STEMCELL Technologies). Both were complemented with 10 ng/mL BDNF and GDNF (Cell Guidance Systems Ltd., Cambridge, UK), 10 nM compound E (Abcam, Cambridge, UK) and 10 nM Trichostatin A (Abcam). Half the medium was changed every other day until the specified maturation time.

2.4. RNA extraction

Sedimented spheres were rinsed once in PBS 1× (Thermo Fischer Scientific Inc.) and then incubated with Gentle Cell Dissociation Reagent (STEMCELL Technologies) for 5 min to disintegrate the alginate capsule. After two PBS 1× washes, total RNA were extracted using the Nucleospin RNA XS kit (Macherey-Nagel GmbH and Co. KG, Düren, Germany). Validation experiments were performed on total RNA extracted from human adult normal cerebral cortex (BioChain Institute Inc., Newark, CA).

2.5. Analysis of neuronal maturation by RT-PCR

A total of 250 ng of RNA was retrotranscribed using the RETROscript reverse transcription kit (Thermo Fischer Scientific Inc.) with oligo(dT) primers, according to the manufacturers' instructions for the 2-step RT-PCR procedure. The PCR reactions were performed using 1 µL of cDNA as template and appropriate forward and reverse primers (Table S1). PCR products were separated by electrophoresis on a 1.5% agarose gel.

2.6. Analysis of adult *MAPT* isoforms by fluorescent RT-PCR

Retrotranscription was performed on 70 ng of RNA, using the Verso cDNA kit (Thermo Fischer Scientific Inc.) and oligo(dT) primers. Primers used in this study are listed in Table S1. The relative proportion of *MAPT* isoforms was then analyzed by fluorescent PCR, using an unlabeled forward primer and a 6-FAM-labelled reverse primer. PCR products were run on a 3500 Genetic Analyzer (Applied Biosystems),

and the electropherograms were analyzed with GeneMapper Software 5.

2.7. Protein extraction and dephosphorylation

Sedimented spheres were rinsed in PBS 1× (Thermo Fischer Scientific Inc.) and then incubated with Gentle Cell Dissociation Reagent (STEMCELL Technologies) for 5 min to disintegrate the alginate capsule. After two PBS 1× washes, spheres were homogenized in Pierce® RIPA buffer (Thermo Fisher Scientific Inc.), supplemented with a cocktail of protease inhibitors (Sigma-Aldrich), using the TissueLyserLT (Qiagen, Hilden, Germany) through high-speed shaking (50 Hz, 2 min) in 1.5 mL microcentrifuge tubes with two 2 mm stainless steel beads. Samples were then spun down, and the lysates collected. After 30 min on ice, and centrifugation (11,300 ×g, 20 min, 4 °C), the supernatant containing soluble proteins collected. Thirty µg of total proteins were then treated with 80 units of lambda protein phosphatase (New England Biolabs, Ipswich, MA) for 3 h at 37 °C in a 50 µL final volume.

2.8. Western blotting

Western blot analyses were performed as described previously (Pons et al., 2017). Briefly, dephosphorylated sample was resolved by a 10% SDS-PAGE simultaneously with 1 µL of Tau Protein ladder (Sigma Aldrich) and then transferred onto nitrocellulose membrane. Membranes were immunoblotted with the primary antibody: Polyclonal Anti-human Tau (Dako Denmark A/S, Glostrup, Denmark) (1:50,000) and revealed using chemiluminescence reagents (ECL Clarity, Bio-Rad Laboratories).

3. Results and discussion

3.1. Generation of iPSC-induced cortical neurons and glial lineage inside Matrigel-coated alginate capsules

In this study, we used iPSCs derived from healthy donors. After dissociation, iPSCs were encapsulated inside Matrigel-coated alginate capsules as previously described for neural stem cells (NSC) in (Alessandri et al., 2016). Capsules were first maintained in mTESR medium to allow colonies to emerge inside the capsules. Then, neural induction of iPSCs was performed by dual-SMAD inhibition until NSCs reached 100% confluency inside the capsules (Feyoux et al., 2012). The efficiency of neural induction was confirmed by assessing *POU5F1* and *OTX-1* mRNA expression by RT-PCR. As expected, we found that the pluripotency gene *POU5F1* was highly expressed in iPSCs and nearly undetected in NSCs (Fig. 1A). On the other hand, the NSC marker *OTX-1* was specifically up-regulated in NSCs as compared to non-induced iPSCs (Fig. 1B). Once NSCs reached confluency, they were then differentiated into cortical neurons. We tested two cell culture media: a commonly used medium (DMEM/F12 – Neurobasal 1:1, D/N) or the BrainPhys medium that has been designed to promote the maturation and synaptic function of iPSC-derived neurons (Bardy et al., 2015). Because previous studies showed that cortical neurogenesis from iPSCs *in vitro* follows the same temporal order as occurs in mammalian cortical development and extends at least as late as day 90 in culture (Espuny-Camacho et al., 2013; Kirwan et al., 2015; Shi et al., 2012), we arbitrarily decided to characterize the identity of iPSC-derived human cortical neurons in our experimental procedure after 15, 20 or 25 weeks of culture. We evaluated the expression of *CALB1* (*Calbindin 1*) and *RELN* (*Reelin*), two specific markers of the upper layer cortical neurons. The upper layer cortical neurons are generated at the latest stages of cortical neurogenesis. *CALB1* is expressed in cortical layers II and III in human cerebral cortex and *RELN* in cortical layer I. As shown in Fig. 1C and D, we detected *CALB1* and *RELN* mRNA expression in iPSC-derived neurons as early as 15 weeks of culture whatever the culture medium

used. The expression of both genes was maintained until 25 weeks. As expected, *CALB1* and *RELN* were not detected at a significant level in iPSCs and NSCs. We also investigated the presence of astrocytes and myelinating oligodendrocytes in our cultures by evaluating *GFAP* (*Glial fibrillary acidic protein*) and *CLDN11* (*Claudin11*) mRNA expression, respectively. We detected *GFAP* expression as soon as 15 weeks of culture in both experimental conditions (Fig. 1E). In contrast, if *CLDN11* expression was readily detected in capsules cultured in BrainPhys medium as soon as 15 weeks, its expression remained absent in those cultured in standard medium even at the extended time point of 25 weeks of differentiation (Fig. 1F). Again, as expected, none of these genes was expressed in iPSC and NSC stages. Altogether, these results indicate that iPSC-induced neurons inside Matrigel-coated alginate capsules were able to differentiate into cortical neurons, including the upper layer cortical neurons that are generated at the latest stages of cortical neurogenesis. Importantly, the presence of oligodendrocytes specifically in BrainPhys-maintained cultures highlights that the BrainPhys neuronal medium improved neuronal maturation compared to the commonly used D/N medium system.

3.2. Development of a quantitative method to measure MAPT adult isoforms: validation in human cerebral cortex

Currently, analyses of the expression of adult *MAPT* mRNA isoforms are mostly based on the sole quantification of the inclusion of exon 10, to determine the 3R/4R ratio, or the inclusion of exons 2 and 3, to evaluate the proportion of 0N, 1N or 2N isoforms. In the present study, we sought to develop a method that would enable the simultaneous analysis of the 6 adult *MAPT* mRNA isoforms individually in a single PCR reaction to examine their relative appearance during neuronal maturation. To this aim, we developed a new assay based on the amplification of *MAPT* transcripts using fluorescent-labelled primers. We designed a set of primers spanning exons 1 and 11 (Fig. 2A) that should enable the amplification of all 6 *MAPT* isoforms. To validate this assay, we performed RT-PCR experiments on mRNA extracted from human adult cerebral cortex. Electropherogram analysis of the PCR-amplified products revealed a 6-peak profile corresponding to the expected sizes of the 6 adult *MAPT* mRNA isoforms (Fig. 2B). We then calculated the relative proportion of the different isoforms from the peak height. We found that the 0N3R and 1N3R isoforms were the most expressed (30.6 and 27.5%), followed by the 0N4R and 1N4R isoforms (22.4 and 17.6%), while the 2N3R and 2N4R isoforms only represented 1% and 0.9% of all isoforms. These data also allowed the determination of the relative proportion of the 0N (53%), 1N (45.1%) and 2N (1.9%) isoforms, and of the 3R (59.1%) and 4R (40.9%). Importantly, similar quantitative data were obtained when analyzing independently the inclusion of exons 2/3 and the inclusion of exon 10 (Fig. S1A, B): the 0N and 1N isoforms were confirmed to be the most expressed (52.9% and 45%, respectively), while the 2N isoforms only represented 2%. Regarding exon 10 splicing, relative quantification of the peaks indicated that the ratio was slightly in favor of the 3R isoforms, with 59.9% of 3R-Tau vs 40.1% of 4R-Tau. These data are fully consistent with previously published studies (Goedert and Jakes, 1990; Leroy et al., 2006) and therefore validate this new assay.

3.3. BrainPhys promotes the expression of the 6 adult MAPT mRNA isoforms in iPSC-induced neurons cultured inside Matrigel-coated alginate capsules

Having validated our assay on adult brain extracts, we then applied our method to evaluate the expression of *MAPT* adult isoforms in iPSC-derived cultures maintained in D/N or in BrainPhys media, over a time-course of maturation of 25 weeks. Consistent with previous studies (Sposito et al., 2015; Verheyen et al., 2015), the analysis of NSCs revealed a single peak profile, corresponding to the expected size of the 0N3R *MAPT* isoform (Fig. 3A, Table 1A). As soon as 15 weeks of

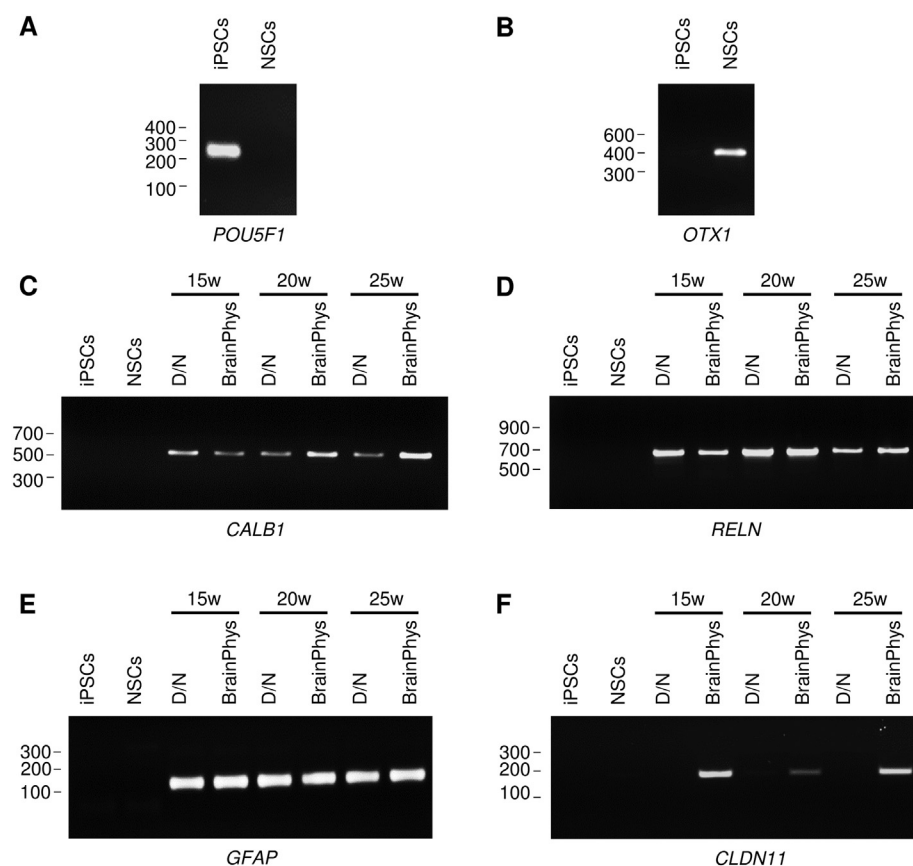


Fig. 1. Molecular characterization of iPSCs, NSCs and iPSC-induced neurons.

RT-PCR analysis on iPSCs, NSCs and differentiated neurons at different time points, using the pluripotency marker *POU5F1* (A), the NSC marker *OTX-1* (B), two specific markers of the upper layer cortical neurons: *CALB1* (C) and *RELN* (D), *GFAP* as an astrocyte marker (E) and the oligodendrocyte-specific marker *CLDN11* (F). Cells were differentiated for 15, 20 or 25 weeks (w), in DMEM/F12 – Neurobasal (D/N) medium or BrainPhys medium.

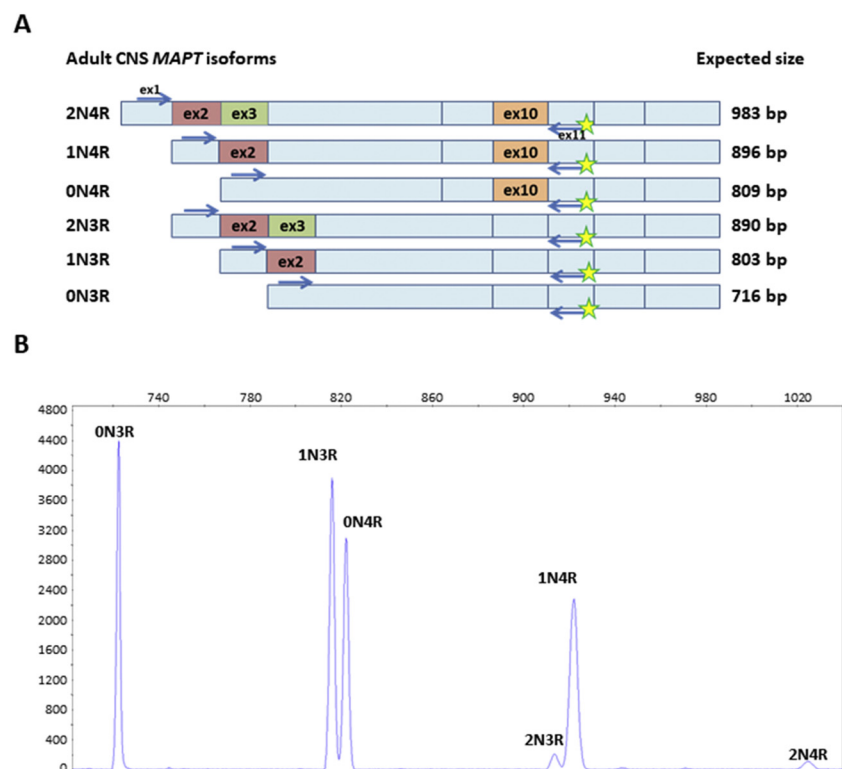


Fig. 2. Detection of the 6 adult *MAPT* mRNA isoforms in brain extracts. (A) Schematic representation of the different *MAPT* isoforms analyzed. The expected sizes of each PCR product were calculated according to the location of the primers spanning exon 1 and exon 11. (B) Each peak corresponds to a different *MAPT* isoform. The Y axis displays fluorescence in arbitrary units, and the X axis indicates the size in bp.

maturation, we detected isoforms including exon 2 (the 1N3R and 1N4R isoforms) and exon 10 (the 0N4R and 1N4R isoforms). Interestingly, BrainPhys-maintained cultures had higher expression levels of the 1N3R, 0N4R and 1N4R isoforms vs. D/N cultures. After 20 weeks of

maturation, we observed a significant increase of the amount of these species, with the appearance of low levels of expression of the 2N3R isoform in both experimental conditions. At the latest time point examined (25 weeks), the expression levels of these adult *MAPT* mRNA

isoforms continued to rise. More importantly, the 2N4R isoform was now detectable in BrainPhys-maintained cultures. Quantitative analysis of the relative proportions of *MAPT* mRNA isoforms in 25 weeks BrainPhys-maintained cultures revealed that the 0N4R and 1N3R isoforms each represented 9.4% of all isoforms, while the 1N4R isoform represented 2.19%. The 2N3R and 2N4R isoforms corresponded to 0.69% and 0.16%, respectively. The 4R-Tau isoforms constituted therefore 11.75% of the *MAPT* transcripts (Table 1B), while the 1N and 2N isoforms accounted for 11.63% and 0.85%, respectively (Table 1C). As was done for brain extracts, we validated these results by independent assessments of the splicing of exon 10 or exons 2/3 (Fig. S1C).

If our experimental procedure allowed the generation of neurons expressing the 6 adult *MAPT* mRNA transcripts, the 0N3R isoform remained predominantly expressed after 25 weeks of maturation (~78%). To exclude that the 0N3R isoforms could “trap” the primers and therefore decrease the efficiency of PCR regarding the lower-expressed transcripts, we therefore sought to analyze *MAPT* isoform expression independently of the 0N3R transcripts. To this aim, we designed two sets of primers (Fig. S2): the first one spanning exons 2 and 11 to analyze precisely the 1N3R, 1N4R, 2N3R, and 2N4R transcripts, and the second one spanning exons 1 and 10 to amplify specifically the 4R-Tau isoforms. Analysis of brain extracts as well as BrainPhys-maintained cultures for 25 weeks confirmed the relative proportions of the different *MAPT* isoforms, further validating our assay.

In order to validate that adult *MAPT* transcripts were efficiently translated in our experimental model, we then assessed Tau isoforms production by western blotting in 25 weeks BrainPhys-maintained spheres. Protein samples were dephosphorylated or not using lambda phosphatase and resolved next to a recombinant Tau ladder containing the 6 Tau isoforms. As shown in Fig. 3B, Tau proteins migrate in multiple bands between 40 and 60 kDa, due to (i) isoform composition and (ii) phosphorylation state. Phosphatase treatment resulted in a shift of Tau species towards lower molecular weights. In agreement with transcript analysis, we found that 0N3R-Tau was the major isoform detected in 25 weeks capsules. However, we detected substantial amounts of 0N4R, 1N3R and 1N4R isoforms. We were unable to observe 2N Tau isoforms. However, as these species comprised < 1% of total Tau, it is possible that their levels were below the limit of detection in our analysis.

Overall, these results showed that iPSC-induced neurons cultured inside Matrigel-coated alginate capsules and maintained for 25 weeks in BrainPhys media, even they did not recapitulate the exact proportion of *MAPT* isoforms observed in the adult human brain, expressed the 6 adult *MAPT* mRNA transcripts, including the 2N3R and 2N4R isoforms. Importantly, when iPSC-derived cultures were maintained in the D/N medium, only 5 isoforms were expressed. The 2N4R isoform was undetectable. Furthermore, at each time point, the mRNA steady-state levels of each isoform were systematically lower than that observed with the BrainPhys medium. Interestingly, consistent with what has been shown in human brain development (Hefti et al., 2018), we first detected a shift in exon 2 and exon 10 expression. The transition in exon 3 was delayed. Although further investigation will be needed to identify the mechanisms behind the better reproduction of *MAPT* splicing when capsules were cultured in BrainPhys medium, these data are fully consistent with recent data indicating that *MAPT* isoform expression correlates with neuronal maturity (Hefti et al., 2018) and the beneficial role of the BrainPhys medium on the maturation state of neurons (Bardy et al., 2015). As well, additional studies will be required to address the contribution of Matrigel-coated capsules to *MAPT* isoform expression. Thanks to our new assay, it would be interesting to compare the expression of the 6 adult *MAPT* mRNA isoforms individually in iPSC-induced neurons cultured inside Matrigel-coated alginate capsules versus other 2D/3D-*in vitro* models using matrigel as support matrix or iPSC-derived human neurons transplanted into mouse brain (Espuny-Camacho et al., 2017). Indeed, in most of these studies, *MAPT* isoform

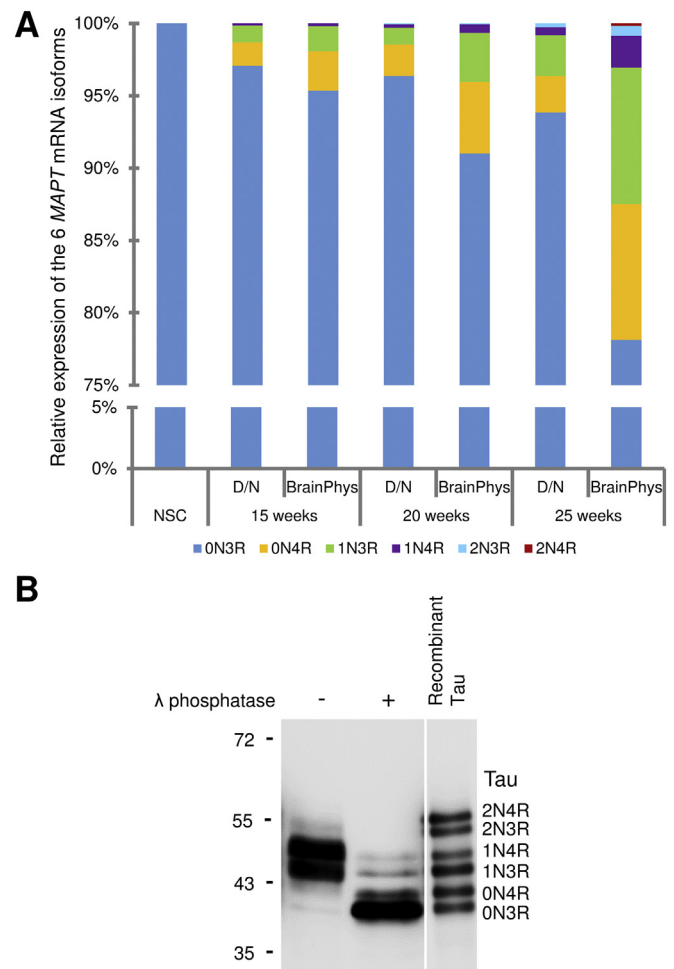


Fig. 3. Expression of adult mRNA and protein *MAPT* isoforms in iPSC-induced neurons.

(A) Representation of the relative expression of the 6 *MAPT* isoforms in iPSC-induced neurons after 15, 20 and 25 weeks of maturation by RT-qPCR. Cells were differentiated for 15, 20 or 25 weeks (w), in DMEM/F12 – Neurobasal (D/N) medium or BrainPhys medium. (B) Western Blot analysis of proteins extracted from 25 weeks neuronal capsules fed with BrainPhys. Treatment with Lambda phosphatase revealed 4 Tau isoforms, corresponding to 1N4R, 1N3R, 0N4R and 0N3R. 2N isoforms remained below detection range.

expression measure was mostly based on the sole quantification of the 3R/4R ratio and/or the proportion of 0N, 1N or 2N isoforms. Following the progression of *MAPT* isoform expression in our experimental system over extended period of time would also be of particular interest.

4. Conclusions

To conclude, we showed that iPSC-induced neurons inside Matrigel-coated alginate capsules displayed specifications of mature cortical cell cultures. More importantly, using a new assay that allowed both the qualitative and the quantitative analysis of all adult *MAPT* mRNA isoforms individually, we demonstrated that BrainPhys-maintained neurons expressed the 6 adult *MAPT* mRNA transcripts after 25 weeks of maturation, making this model highly suitable for modeling Tau pathology and therapeutic purposes.

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.scr.2019.101541>.

Declaration of Competing Interest

Pierre Nassoy and Maxime Feyeux have recently co-founded

Table 1
Quantification of the relative expression of the 6 adult *MAPT* mRNA isoforms.

A						
	0N3R	0N4R	1N3R	1N4R	2N3R	2N4R
NSCs	100.00 (± 0.00)					
15w-D/N	97.09 (± 0.07)	1.60 (± 0.13)	1.17 (± 0.04)	0.14 (± 0.02)		
15w-BrainPhys	95.36 (± 0.22)	2.70 (± 0.47)	1.75 (± 0.22)	0.20 (± 0.03)		
20w-D/N	96.37 (± 0.11)	2.17 (± 0.08)	1.17 (± 0.03)	0.22 (± 0.01)	0.07 (± 0.00)	
20w-BrainPhys	91.00 (± 0.60)	4.94 (± 0.37)	3.40 (± 0.64)	0.58 (± 0.10)	0.07 (± 0.07)	
25w-D/N	93.85 (± 1.02)	2.51 (± 0.35)	2.83 (± 0.44)	0.56 (± 0.12)	0.26 (± 0.11)	
25w-BrainPhys	78.12 (± 0.49)	9.40 (± 0.56)	9.44 (± 0.28)	2.19 (± 0.17)	0.69 (± 0.04) I	0.16 (± 0.01)
B						
			3R			4R
NSCs			100.00			–
15w-D/N			98.26			1.74
15w-BrainPhys			97.10			2.90
20w-D/N			97.61			2.39
20w-BrainPhys			94.48			5.52
25w-D/N			96.94			3.06
25w-BrainPhys			88.25			11.75
C						
	0N		1N			2N
NSC	100.00					
15w-D/N	98.69		1.31			
15w-BrainPhys	98.06		1.94			
20w-D/N	98.54		1.39			0.07
20w-BrainPhys	95.95		3.98			0.07
25w-D/N	96.35		3.39			0.26
25w-BrainPhys	87.52		11.63			0.85

MAPT mRNA isoforms were quantified either individually (A), grouped depending on inclusion of exon 10 (B), or inclusion of exons 2 and 3 (C). (A) The data represent the mean ± standard deviation (SD) from at least two independent experiments.

TreeFrog Therapeutics SAS, a biotechnology start-up specializing in encapsulation of pluripotent stem cells.

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