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Supplemental Information

Increased 4R-Tau Induces Pathological Changes in a Human-Tau Mouse Model

Kathleen M. Schoch, Sarah L. DeVos, Rebecca L. Miller, Seung J. Chun, Michaela Norrbom, David F. Wozniak, Hana N. Dawson, C. Frank Bennett, Frank Rigo, and Timothy M. Miller

Supplemental Figures and Legends

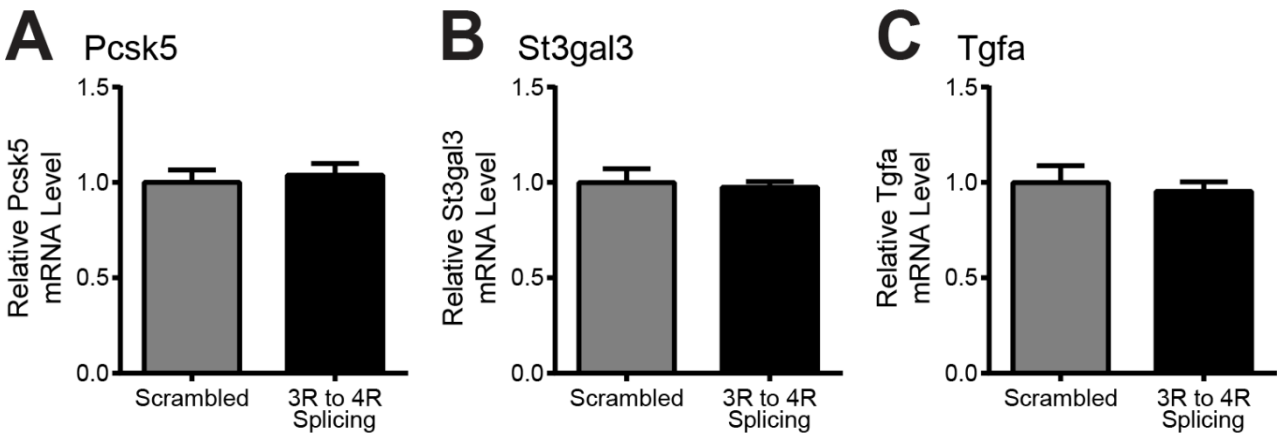

Figure S1: Exclusion of off-target mRNA alteration by 3R to 4R *MAPT* splicing ASO within the mouse genome. (Related to Figure 1)

NCBI BLAST analysis of the scrambled and 3R to 4R human *MAPT* targeting ASO sequences was performed against the mouse genome to identify potential off-target mRNAs. While no mouse genome sequences produced a significant alignment with the ASO query (i.e. E value < 0.05), the best aligned mouse mRNAs by maximum score were analyzed in our treated hTau samples via quantitative real-time PCR. These genes included: **A)** Pcsk5 (proprotein convertase subtilisin/kexin type 5), **B)** St3gal3 (ST3 beta-galactoside alpha-2,3-sialyltransferase 3), and **C)** Tgfa (transforming growth factor, alpha). 3R to 4R *MAPT* splicing ASO treatment did not significantly alter the mRNA profile of Pcsk5, St3gal3, or Tgfa, suggesting that the increased pathology and functional deficits identified in treated hTau mice is not likely attributed to off-target effects. A fourth gene, Serpinb9c (serine (or cysteine) peptidase inhibitor, clade B, member 9c), was also analyzed but not appreciably detected in brain lysates. Based on mRNA analysis through NCBI BLAST and UCSC Genome Browser, we can also confirm that the ASO alignments are not predicted to occur at alternative splice sites, excluding the possibility that our ASOs are affecting off-target isoform expression. Data are expressed as mean + SEM relative to scrambled controls and analyzed by unpaired t-test.

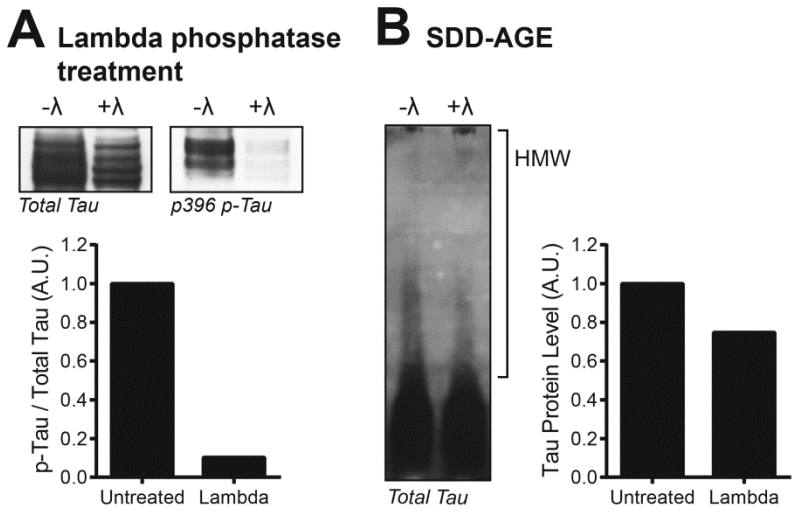

Figure S2: Contribution of phosphorylation to high molecular weight tau species in SDD-AGE analysis. (Related to Figure 2)

A) To identify the contribution of phosphorylation status to the higher molecular weight shift seen with 3R to 4R splicing ASO treatment, an hTau 3R to 4R splicing ASO sample was either untreated (-λ) or incubated with lambda phosphatase (+λ) to dephosphorylate tau and run on an SDS-PAGE gel for analysis of total tau (HT7) and phospho-tau (p396 p-Tau). Densitometric analysis of phospho-tau normalized to total tau confirmed successful dephosphorylation with lambda phosphatase treatment. **B)** The untreated and lambda-treated hTau 3R to 4R splicing ASO sample was then analyzed via SDD-AGE. Semi-quantitation of the high molecular weight (HMW) banding area revealed a small reduction in HMW tau with lambda phosphatase treatment, suggesting phosphorylation contributes to some but not all of the shift in tau species. Thus, increased HMW seen with increased 4R-tau in hTau mice (Figure 3) is likely due to both phosphorylated and oligomeric tau. Data are expressed as a mean value; A.U. arbitrary units.

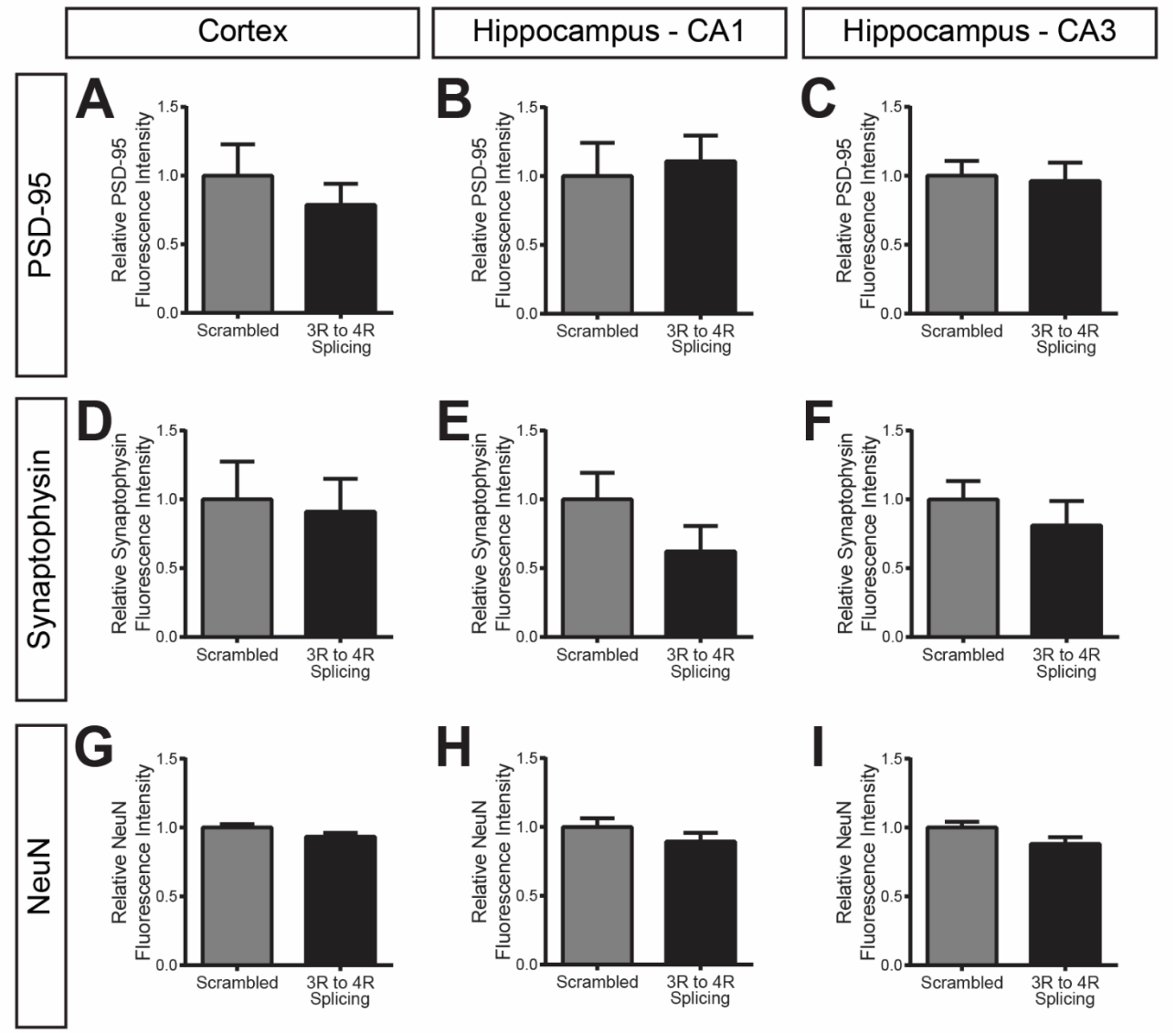

Figure S3: Immunohistochemical analysis of synapses and neurons of ASO-treated hTau mice. (Related to Figure 3)

Contralateral tissue sections obtained from hTau mice treated with scrambled ASO (n=5) and 3R to 4R *MAPT* splicing ASO (n=7) were evaluated for gross synaptic structure using the antibodies, post-synaptic density-95 (PSD-95) and synaptophysin, and for neuronal density by the antibody, NeuN. Markers of **A-C)** PSD-95, **D-F)** synaptophysin, or **G-I)** NeuN were not statistically different between treatments as measured in the cortex and CA1 and CA3 regions of the hippocampus. Fluorescent immunoreactivity was quantified as a mean fluorescent intensity + SEM and illustrated relative to scrambled ASO treatment. An unpaired t-test was used to compare treatments.

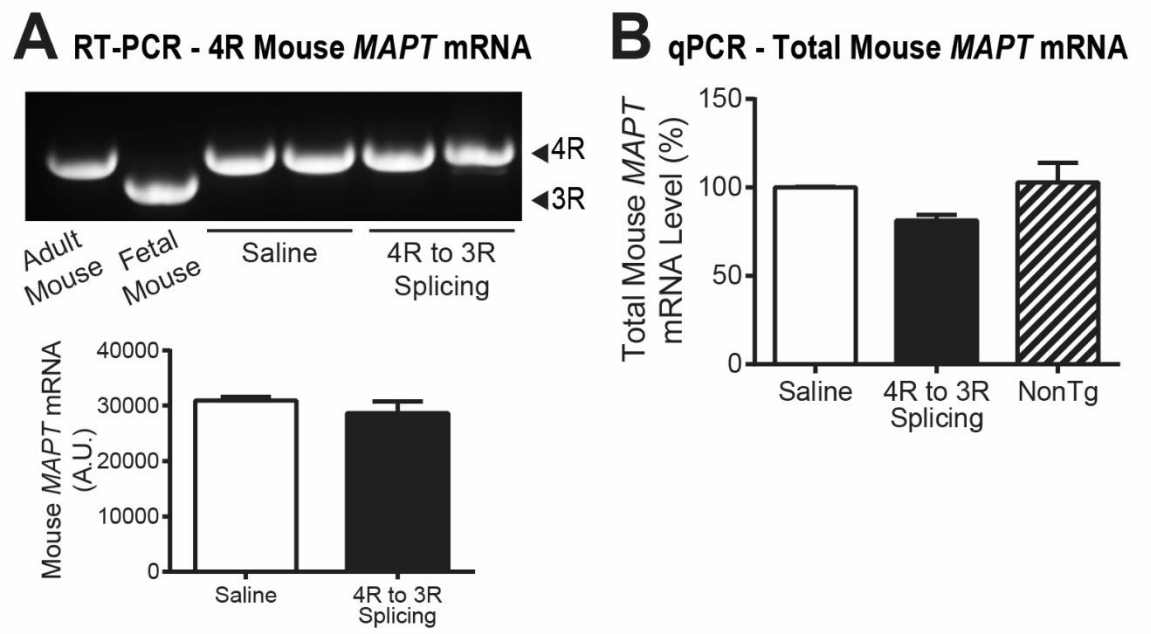

Figure S4: Specificity of 4R to 3R human *MAPT* splicing ASO in Tau N279K mice. (Related to Figure 4)

A) RT-PCR analysis of mouse *MAPT* mRNA in 4R to 3R *MAPT* splicing ASO-treated samples revealed no change in exon 10 splicing of mouse *MAPT* mRNA compared to saline treatment. Adult mouse and fetal mouse brain samples were included as size controls for 4R and 3R *MAPT*, respectively. Total mouse *MAPT* (i.e. 4R) mRNA bands were measured by densitometric analysis and expressed as arbitrary units (A.U.). **B)** Similarly, total mouse *MAPT* mRNA levels measured by qPCR analysis were not significantly altered by treatment, confirming splicing specificity for human *MAPT*. Total *MAPT* mRNA data are expressed as a percent relative to saline treatment (mean + SEM).

Supplemental Experimental Procedures

Experimental animals

Human tau transgenic mice

As described in Andorfer et al., 2003, generation of the human tau (hTau) transgenic mouse line involved insertion of a human tau cDNA-containing PAC vector into female donor mice. The resulting mice (known as 8c mice) were bred to mouse tau knockout (mTau-/-) mice and backcrossed to a C57BL/6J background to obtain mice exclusively expressing human tau. RT-PCR analysis of mouse brains using human-specific primers confirmed the presence of *MAPT* isoforms 0N, 1N, and 2N as well as isoforms with and without exon 10 (4R and 3R, respectively) (Andorfer et al., 2003). hTau transgenic mice were purchased (Jackson Laboratories, Bar Harbor, ME) and maintained in-house through breeding between hTau+/- mTau-/- mice and mTau-/- mice. Mice were housed in a controlled, single-barrier facility with 12:12 light:dark photoperiod and chow diet and water *ad libitum*. Human tau genotyping was performed by PCR amplification of tail DNA using the primers 5'-ACT TTG AAC CAG GAT GGC TGA GCC C-3' and 5'-CTG TGC ATG GCT GTC CAC TAA CCT T-3'.

Human N279K mutant tau transgenic mice

Human N279K mutant tau mice as described in Dawson et al., 2007 were created by microinjection of a human *MAPT* minigene construct, containing both intron and exon sequences and N279K FTDP-17 mutation within exon 10, into mice of mixed C57BL/6//SJL background to produce transgenic mice expressing human tau^{N279K} under the *MAPT* gene promoter. Human-specific RT-PCR primers amplifying exons 2 and 3 and exon 10 were used to demonstrate the exclusive presence of 2N4R *MAPT* mRNA (441 bp) in N279K transgenic mice, albeit at levels well below mouse tau (Dawson et al., 2007). Thus, expression of the N279K-mediated mRNA splicing aberration under the endogenous human tau promoter in this model closely parallels the genetics of human FTDP-17. Tau N279K mice were obtained from Duke University and maintained at Washington University as heterozygotes by breeding Tau N279K transgenic mice with C57BL/6 mice (Jackson Laboratories). Mice positive for the N279K human tau transcript were identified by PCR amplification of tail DNA using the same primer sequences listed for hTau genotyping.

Antisense oligonucleotides (ASOs)

ASO sequences used for experiments are as follows. For 3R to 4R *MAPT* splicing in hTau mice: 5'-GGC GCA TGG GAC GTG TGA-3'; and scrambled control: 5'-TCA TTT GCT TCA TAC AGG-3'. For 4R to 3R *MAPT* splicing in Tau N279K mice: 5'-CAG ATC CTG AGA GCC CAA-3'; and scrambled control: 5'-CCT TCC CTG AAG GTT CCT CC-3'. Although similar in structure and modification to the above splicing oligonucleotides (Rigo et al., 2014), the 4R to 3R *MAPT* splicing ASO used in hTau transgenic mice targeted a different region of the human tau sequence (5'-GGA CGT GTG AAG GTA CTC-3'). Due to the ASO specificity for human *MAPT*, mouse tau remains unaltered. An *in silico* analysis was performed with the 3R to 4R and 4R to 3R *MAPT* ASOs to ensure their specificity toward human tau mRNA. The program Bowtie (Langmead et al., 2009) was used to determine the predicted off-targets for the *MAPT* ASOs in the mouse transcriptome (pre- and m-RNA). This analysis confirmed that the *MAPT* ASOs do not bind any RNA other than *MAPT* with full. Gene downregulation events are not anticipated given that ASOs that modulate splicing do not induce RNaseH- or Ago2-mediated mRNA degradation.

Intraventricular delivery of ASOs

Mice were anesthetized by 2-3% inhalant isoflurane and stabilized within a stereotaxic head frame (Kopf, Tujunga, CA). While receiving constant isoflurane flow, a scalp incision was made and a subcutaneous pocket created along the left side of the body. The osmotic pump, with plastic tubing and catheter attachment, was then placed within the pocket. The metal catheter was positioned -1.1mm M/L, -0.5mm A/P, -2.5mm D/V from bregma (Franklin and Paxinos, 2013) for insertion into the intraventricular space and directly driven into the skull. A small amount of super glue (Loctite, Westlake, OH) placed on the plastic cannula base ensured immobilization of the catheter within the lateral ventricle. The incision was closed and mice were placed on a 37°C warming recovery pad until ambulatory. Following the surgical procedure, all mice were individually housed.

Osmotic pumps were assembled according to manufacturer's instructions (ALZET) and allowed to equilibrate in a 37°C water bath overnight prior to surgical implantation. Drug delivery occurred over the course of 28 days at a rate of 6 µl of saline or ASO per day. Intraventricular infusion of ASOs previously has been shown to result in widespread distribution of the compound throughout the cortex, hippocampus, thalamus, brainstem, and cerebellum (DeVos et al., 2013, Kordasiewicz et al., 2012) with both neuronal and non-neuronal uptake (Kordasiewicz et al., 2012); therefore, we anticipated adequate delivery of ASOs throughout the CNS of experimental mice. The dose of ASO for splicing studies was empirically determined to achieve effective splicing without overt adverse effects: 15 µg/day for 3R to 4R *MAPT* splicing ASO and 25 µg/day for 4R to 3R *MAPT* splicing ASO. Scrambled ASOs were given at the identical rate and dose for respective experiments. Behavioral assessments and euthanasia occurred two months following the cessation of ASO infusion.

mRNA isolation and analysis following MAPT splicing

mRNA was extracted from tissues using RNeasy® Mini Kit (Qiagen, Venlo, Netherlands) and its concentration determined by Nanodrop spectrophotometer (Thermo Scientific, Waltham, MA). *MAPT* mRNA levels from the hTau mouse model were readily detectable by standard quantitative real-time PCR methods using EXPRESS One-Step Superscript qRT-PCR Universal Kit (Invitrogen, Carlsbad, CA) for reverse transcription and amplification and ABI PRISM 7500 Fast Real-Time PCR System for comparative analysis by the $\Delta\Delta C_t$ method (Applied Biosystems, Waltham, MA). Given that human N279K *MAPT* mRNA levels were much lower in comparison to endogenous mouse MAPT mRNA (Dawson et al., 2007), a modified RT-PCR protocol was used. Isolated RNA from Tau N279K mice was reverse transcribed (SuperScript II Reverse Transcriptase; Invitrogen). The cDNA was then run in a PCR reaction using a PCR enhancer system kit (Invitrogen) and Platinum *Taq* DNA Polymerase (Invitrogen) with radiolabeled ^{32}-P-dCTP (PerkinElmer, Waltham, MA). PCR products were digested using *Hinc*II to discriminate mouse versus human tau products. The radiolabeled PCR samples were run on a 6% native polyacrylamide gel under denaturing conditions, allowing for size separation of human 4R (496bp digested; 745bp undigested) and 3R (403bp digested; 652bp undigested) *MAPT* cDNA, and detected on a STORM optical scanner (GE Healthcare Bio-Sciences, Pittsburgh, PA). Individual 3R and 4R bands were quantified by ImageJ (National Institutes of Health, Bethesda, MD) densitometric analysis. The signal intensity of each cDNA band was normalized according to its G+C content. The extent of exon 10 inclusion was calculated as a percentage of the total amount of spliced mRNA.

Primer/probe sequences used for detection of total human *MAPT* mRNA following 3R to 4R *MAPT* splicing treatment were: forward 5'-AGA AGC AGG CAT TGG AGA C- 3', reverse 5'-TCT TCG TTT TAC CAT CAG CC-3', probe 5'-/56-FAM/ACG GGA CTG GAA GCG ATG ACA AAA /3IABkFQ/-3'; for human 4R *MAPT* mRNA: forward 5'-CAT GCC AGA CCT GAA GAA TG-3', reverse 5'-GAC TGG ACG TTG CTA AGA TC-3', probe 5'-/56-FAM/ CCA CTG AGA ACC TGA AGC ACC AGC /3IABkFQ/-3' (Integrated DNA Technologies, Coralville, IA). As an internal control, GAPDH primer/probe sequences were: forward 5'-TGC CCC CAT GT TGT GAT G-3', reverse 5'-TGT GGT CAT GAG CCC TTC C-3', probe 5'-/56-FAM/ AAT GCA TCC TGC ACC ACC AAC TGC TT /3IABkFQ/-3' (Integrated DNA Technologies). For hTau mice treated with a 4R to 3R *MAPT* splicing ASO, total human *MAPT* mRNA within brain and spinal cord tissue was detected by the following primer/probe sequences: forward 5'-AAG ATT GGG TCC CTG GAC AAT-3', reverse 5'-AGC TTG TGG GTT TCA ATC TTT TTA TT-3', probe 5'-TTA ATT ATC TGC ACC TTC CCG CCT CC-3'; for human 4R *MAPT* mRNA: forward 5'-CAC TGA GAA CCT GAA GCA CC-3', reverse 5'-GGA CGT TGC TAA GAT CCA GCT-3', probe 5'-TTA ATT ATC TGC ACC TTC CCG CCT CC-3'; and for GAPDH internal control: forward 5'-GGC AAA TTC AAC GGC ACA GT-3', reverse 5'-GGG TCT CGC TCC TGG AAG AT-3', and probe 5'-AAG GCC GAG AAT GGG AAG CTT GTC ATC-3'.

Primer sequences used for detection of 3R and 4R *MAPT* mRNA isoforms in Tau N279K mice were: forward 5'-AAC GAA GAT CGC CAC ACC-3' and reverse 5'-CGA CTT GTA CAC GAT CTC C-3', complimentary to only human tau. To confirm the human specificity of 4R to 3R *MAPT* splicing ASO, mouse *MAPT* mRNA obtained from treated Tau N279K mice was amplified by OneStep RT-PCR kit (Qiagen) using primers: forward 5'-GAA CCA CCA AAA TCC GGA GA -3', reverse 5'-CTC TTA CTA GCT GAT GGT GAC-3' and probe 5'-/56-FAM/CCA AGA AGG TGG CAG TGG TCC/3IABkFQ/- 3'; and for RT-PCR: forward 5'-GAA GAT CGC CAC ACC TCG-3' and reverse 5'-GGT GAC TTA TAC ACA ATT TCT G-3'. RT-PCR products were visualized on an agarose gel, using ImageJ to semi-quantitatively measure the 3R and 4R band densities.

Tissue homogenization and tau protein analyses by immunoblot and ELISA

Brain tissue designated for protein analysis (temporoparietal region ipsilateral to the catheter) was homogenized in RAB buffer (750mM sodium chloride, 100mM MES, 20mM sodium fluoride, 1mM EDTA, 1mM sodium orthovanadate, 0.5mM magnesium sulfate heptahydrate) supplemented with protease inhibitor cocktail (Sigma, St. Louis, MO) and phosphatase inhibitors (PhosSTOP; Roche, San Francisco, CA) and centrifuged at 21,000 x g for 15 minutes at 4°C. For tau fractionation experiments, brain tissue was homogenized and ultracentrifuged in RAB buffer, RIPA buffer (150mM NaCl, 50mM Tris, 0.5% deoxycholic acid, 1% Triton X-100, 0.5% sodium dodecyl sulfate, 25mM EDTA, pH 8.0), and 70% formic acid as previously described in detail (Yamada et al., 2011). RAB- and RIPA-soluble supernatants were analyzed by BCA protein assay and formic acid fractions by Bradford assay (Thermo Scientific) for protein concentration.

Immunoblots for total tau and tau isoforms were used to visualize tau protein expression and tau isoform changes following ASO treatment in mice. Protein homogenates were run on 12% SDS-PAGE gels, transferred to PVDF membrane, and blocked for 1 hour in 5% dry milk/1X PBS/0.05%Tween-20. Following overnight incubation in primary antibody, membranes were incubated in HRP-conjugated secondary antibody and detected using ECL 2 substrate (Thermo Scientific) on G:Box Chemi XT4 Imaging System (Syngene, Frederick, MD). Primary antibodies were diluted in 5% dry milk/1X PBS/0.05% Tween-20 and included total human tau (mouse monoclonal HT7, 1:1000, Thermo Fisher Scientific Cat# MN1000 RRID: AB_223454), 3R-tau (RD3 1:1000; Millipore Cat# 05-803 RRID: AB_310013), and 4R-tau (RD4 1:500; Millipore Cat# 05-804 RRID: AB_11211556) with anti-mouse IgG HRP secondary (1:5000; GE Healthcare Cat# NA931-1ml RRID: AB_772210). Membranes were washed and re-probed for GAPDH (rabbit monoclonal 1:5000; Cell Signaling Technology Cat# 2118L RRID: AB_1031003) and anti-rabbit IgG HRP secondary antibody (1:5000; GE Healthcare Cat# NA934-1ml RRID: AB_772206) for a loading control.

Quantification of total human tau was performed by enzyme-linked immunosorbent assay (ELISA) with tau-5 (20 µg/mL; Millipore Cat# 577801-100UG RRID: AB_212534) coating antibody and human-specific HT7 (0.3 µg/mL, biotin-conjugated; Thermo Fisher Scientific Cat# MN1000B RRID: AB_223453) capture antibody. Following an hour incubation in blocking reagent (4% bovine serum albumin (BSA) diluted in 1X PBS), recombinant human tau standard (2N4R; rPeptide, Bogart, GA) and protein samples were

diluted in ELISA buffer (300mM Tris, 0.25% BSA, 1X protease inhibitor cocktail) and applied to half-well plates for overnight incubation. Human tau was detected using HT7 capture antibody followed by streptavidin poly-HRP40 conjugate (1:4000; Fitzgerald, Acton, MA) and 3,3',5,5'-tetramethylbenzidine liquid substrate, Super Slow (Sigma) reagent and read by microplate spectrophotometer (Biotek Epoch, Winooski, VT).

Detection of tau by SDD-AGE

In preparation of SDD-AGE analysis, occipital brain lysate was thawed on ice and a 1.5% agarose gel was prepared by dissolving agarose in buffer G (20mM Tris-Base, 200mM glycine) with the addition of sodium dodecyl sulfate (SDS) to a final concentration of 0.02%. Brain protein lysate (100 µg) was incubated with 0.02% SDS sample buffer at room temperature for 7 minutes prior to loading. Gel electrophoresis was done in Laemmli buffer (Buffer G with 0.1% SDS) at 35V for 14 hours. Protein was then transferred via capillary action using 20 pieces of thick Whatmann (GB 005) and 8 pieces medium Whatmann (GB 003) to Immobilon PVDF membrane (Millipore) at 4°C for 36 hours. Membranes were blocked in 3% BSA 1X TBS-Tween 20 (TBS-T) for 1 hour and then probed for total tau using rabbit polyclonal anti-tau ab64193 (1:4000; Abcam Cat# ab64193 RRID: AB_1143333) overnight at 4°C. Membranes were washed three times with 1X TBS-T, probed with goat anti-rabbit HRP (1:2000; Bio-Rad Laboratories, AbD Serotec Cat# 170-6515 RRID:AB_11125142) for 1.5 hours at room temperature, and washed three times with 1X TBS-T. Membranes were developed using ECL (Thermo Scientific Pierce) for 3 minutes and exposed to film (GE Healthcare Bio-Sciences). To re-probe for phosphorylated tau, the blot was incubated with PHF-1 (1:5000; a gift from P. Davies, The Feinstein Institute for Medical Research; New York; USA Cat# PHF1 RRID: AB_2313687) diluted in 3% BSA in 1X TBS-T with 0.01% sodium azide overnight at 4°C. Once sufficiently washed in 1X TBS-T, the blot was incubated with goat anti-mouse HRP (1:2000; Bio-Rad Laboratories, AbD Serotec Cat# 170-6516 RRID: AB_11125547) and developed in ECL as previously described above.

Lambda phosphatase treatment

Protein lysate (120 µg) was incubated at 30°C for 1.5 hours with 1200 units of lambda phosphatase (New England BioLabs) to dephosphorylate tau. Following incubation, samples were immediately placed on ice and prepared for SDS-PAGE analysis. Lysates (20 µg) were boiled at 95°C for 7 minutes, cooled to room temperature, loaded onto a 4-12% Bis-Tris polyacrylamide gel (NuPAGE, Life Technologies) and run at 120V. Proteins were transferred to PVDF membrane and detection of total tau (HT7, 1:2500) and phospho-tau (p396 p-Tau, 1:2500; Millipore Cat# AB9658 RRID:AB_11213701) occurred overnight at 4°C. Goat anti-mouse IRDye680CW (1:2000; LI-COR Biosciences Cat# 926-32220 RRID: AB_621840) and goat anti-rabbit IRDye800CW (1:2000; LI-COR Biosciences Cat# 926-32211 RRID: AB_621843) secondary antibodies were used to detect total tau and phospho-tau, respectively. Membranes were visualized on an Odyssey LI-COR imaging system (LI-COR Biosciences, Lincoln, NE). SDD-AGE analysis was performed as described in "Experimental Procedures."

Histology

Immunohistochemistry of phosphorylated tau

Fixed, frozen brain tissue contralateral to the catheter placement was sectioned on an HM 430 freezing microtome (Thermo Scientific) at a thickness of 50µm. Serial coronal sections taken approximately 600 µm apart were placed in cryoprotectant solution (30% ethylene glycol, 15% 0.2M phosphate buffer, and 15% w/v sucrose) and stored at -20°C until use. For identification of phosphorylated tau in hTau brain tissue, free-floating tissue were rinsed in PBS. Sections were then incubated in 0.3% v/v hydrogen peroxide to quench endogenous peroxidases, washed, and blocked in 3% nonfat dry milk diluted in PBS containing 0.25% Triton-X. Sections were incubated at 4°C overnight in mouse monoclonal primary antibody (AT8 biotin-conjugated, 1:500; Thermo Fisher Scientific Cat# MN1020B RRID: AB_223648) to detect phosphorylated tau. The biotinylated signal was amplified by incubation in an avidin-biotin solution (Vectastain Elite ABC kit; Vector Laboratories) and detected using diaminobenzidine.

Tissue sections were mounted onto slides and dried overnight. Slides were dehydrated in an ethanol gradient and coverslipped using Cytoseal™ XYL mounting media (Thermo Scientific). Stained tissues were imaged on Olympus NanoZoomer 2.0-HT microscope system (Hamamatsu, Hamamatsu City, Japan) and captured using NDP.scan 2.5 and NDP.view software (Hamamatsu). Image analysis was performed in a blinded fashion using ImageJ in which regions of positive immunoreactivity were identified above an assigned pixel size and threshold value that were kept constant across all treatment groups for each brain area.

Immunofluorescence imaging and analysis of neuronal and synaptic markers

Free-floating tissue sections were rinsed in 1X Tris-buffered saline (TBS) prior to blocking in 5% normal horse serum (NHS) diluted in TBS containing 0.1% Triton X-100. Sections were incubated in primary antibody (NeuN 1:1000, Millipore Cat# MAB377B RRID: AB_177621; synaptophysin 1:1000, Cell Signaling Technology Cat# 5461S RRID: AB_10698743; PSD-95 1:1000, Millipore Cat# AB9708 RRID: AB_2092543) at 4°C overnight and, following an additional blocking incubation, were incubated with fluorescently tagged secondary antibody (anti-rabbit DyLight 488 1:250, Thermo Fisher Scientific Cat# SA5-10038 RRID: AB_2556618 or anti-rabbit AlexaFluor 594 1:250, Jackson ImmunoResearch Labs Cat# 711-585-152 RRID: AB_2340621). Sections were mounted and sealed with Fluoromount media (Southern Biotech, Birmingham, AL). The Olympus NanoZoomer 2.0-HT

microscope system was used for fluorescent image analysis with quantification in ImageJ. For CA1 and CA3 regions, a rectangle was drawn over the region of interest (ROI) and duplicated across multiple images for equivalent ROIs. For cortical measurements, a region was manually drawn around the entire cortex. The mean fluorescence intensity (MFI) and integrated density measurements were recorded from ImageJ and corrected for background fluorescence using the formula: Corrected MFI_{CA1} = ($IntDen_{ROI}$ -($Area_{ROI}$ * $MFI_{Background}$))/ $Area_{ROI}$.

Seizure monitoring

For monitoring of PTZ seizure activity, hTau mice were placed in a clean cage and recorded for 15 min following PTZ injection. Videos were later viewed to record the time at which a seizure stage was reached. Stages were characterized by immobility (stage 1); spasm, twitch, or tremor (stage 2); tail extension (stage 3); forelimb clonus (stage 4); generalized clonus (stage 5); jumping/running seizures (stage 6); tonic extension (stage 7); and death (stage 8) (DeVos et al., 2013). Video recording and viewing was done under blinded conditions such that genotype and treatment were unknown to the observer.

Nesting activity

Mice were individually housed and previous nesting material was removed. An intact 3.0 g nestlet (Ancare, Bellmore, NY) was placed within the cages. Approximately 14 h later, following the nocturnal period, the resulting nest was photographed for qualitative scoring and any untorn nestlet was weighed. All nest images and weights were analyzed by an individual blinded to genotype and treatment.

Statistics

Quantifiable mRNA and protein expression data for both 3R to 4R and 4R to 3R splicing experiments were evaluated by one-way ANOVA with Dunn's multiple comparison *post hoc* analysis. A Kruskal-Wallis one-way ANOVA with Dunn's *post hoc* was used to analyze categorical nestlet scores and seizure severity scores induced by PTZ; however, an unpaired t-test was used to evaluate nesting activity by untorn nestlet weight and a repeated measures two-way ANOVA (treatment x time) with Bonferroni *post hoc* was performed on seizure latency data. Histological quantifications of phosphorylated tau used a one-way ANOVA with Dunn's *post hoc* for multiple group comparisons. In select experimental analyses, saline- and scrambled-treated controls were not statistically different and were subsequently pooled for comparison with splicing treatments. A value of $p<0.05$ was deemed significant for all analyses.

Supplemental References

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