Supplementary Data

Tryptamine-Induced Tryptophanyl-tRNA^{trp} Deficiency in Neurodifferentiation and Neurodegeneration Interplay: Progenitor Activation with Neurite Growth Terminated in Alzheimer’s Disease Neuronal Vesicularization and Fragmentation

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Fig. S1. Phase contrast of epithelioids lacking neurites in old SH-SY5Y (-tryptamine) and during tryptamine insult (20 μg/ml 24 h, epithelioid monolayer). Note absence of satellite clusters, satellite-epithelioids (+tryptamine).

Fig. S2. Congo red/hematoxylin staining of satellites adhered to epithelioids and matrix and transitional forms. Small (blue arrows) and large (black arrows) epithelioids (-tryptamine). Note, asymmetric telophase phenotype (red curved arrow). Matrix-adhered satellites (upper inset), satellites adhered to epithelioids (thin arrows) and transitional satellite-to-epithelioid phenotype (low inset), arrows show non-even plasma membrane (+tryptamine).
Fig. S3. Congo red/hematoxylin staining of tryptamine-stimulated satellite clustering. (-tryptamine) epithelioid phenotype (5.5 months), single satellite (arrow); (+tryptamine) 5-day monolayer, 20\(\mu\)g/ml, 6 d. Satellite clustering (arrow).

Fig. S4. Congo red/hematoxylin staining of tryptamine-treated epithelioids adhered to satellite clusters. Epithelioids were treated with 6\(\mu\)g/ml tryptamine, 22 d (no tryptamine withdrawal). Epithelioids without satellites (black arrows), satellite clusters (red arrows), single satellites (blue arrows).

Fig. S5. Neuronal/fibromuscular phenotype (Congo red/hematoxylin staining). Epithelioid treatment/withdrawal (20\(\mu\)g/ml tryptamine, 24 d; withdrawal, 5.5 m; neurite length 1.3 mm (arrows). Re-treatment (20–40\(\mu\)g/ml tryptamine, 7 d).
Fig. S6. Neuronal phenotype after tryptamine treatment/withdrawal. Elongated neurite (red arrows).

Fig. S7. Tryptamine-treated neurons after tryptamine treatment/withdrawal of SH-SY5Y (live, 49th d). Cells survived 50–100 μg/ml tryptamine treatment as in Fig. 1D. Note, phase-bright cells, metaphase plate (M, inset).
Fig. S8. Phase contrast microscopy of subline with phenotype of differentiation after tryptamine stimulus (Fig. S7 continuation). Micrographs of live cells cultivated in tryptamine-free medium for 2 m. Neuronal-Schwannian-fibromuscular-phenotype. Note that no neuroblasts after tryptamine withdrawal (2 m).

Fig. S9. NF-M immunoreactivity occasionally detected in neurons of untreated aged SH-SY5Y culture. NF-M-short neurites outgrowing from spheroids (red arrowheads). NF-M-negative spheroids lacking processes (white thick arrows), neuroblasts (white thin arrows) and satellite (black arrow) in untreated cells. Phase contrast of live cells (cloned from single spheroids) during tryptamine treatment (20 μg/ml, 18 d; no withdrawal). Note that processes outgrow from epithelioids (black arrowheads).
Fig. S10. Tau in old SH-SY5Y mixed culture comprising epithelioids (arrows), spheroids (arrowheads) and neuroblasts with processes (thin arrows) and after tryptamine treatment/withdrawal. Satellites (arrowheads), possible asymmetric division (curved arrows), vesicles (open arrows). Putative pathway: epithelioid adhered satellite-to-neuroblast (neuroblast repopulation) and/or opposite direction (epithelioid population): neuroblast-to-satellite-to-epithelioid (curved arrows, left low).

Fig. S11. Tau in axons (enlarged, right low) and vesicles (arrows) at neuroblasts and epithelioids after tryptamine treatment/withdrawal. Mitosis (arrowheads). Note adjacent possibly daughter cells of different shapes with and without vesicles in asymmetric mitosis (inset). Epithelioids without vesicles (white arrows), satellites adhered and released (open arrows); dendrites are tau-negative (enlarged, right low).
Fig. S12. The increased numbers of p-tau-plasma membrane vesicles (inset) and satellites adhered to epithelioids (arrowheads) during tryptamine insult (20 μg/ml, 24 d, no withdrawal) compared to control.

Fig. S13. GFAP differential immunostaining. GFAP increase in satellites after tryptamine stimulus (insets, thick arrows). GFAP(−) small epithelioids (blue arrows). GFAP(−) and GFAP(+) processes outgrowing from the same epithelioid. Cell cluster associated with broken neurite (inset, red arrows). Phenotypically SC (green arrow). Vesicles (arrowheads).
Fig. S14. Vimentin (mAb V-9) cells (arrows) wrap around neurite outgrowing from epithelioid after tryptamine treatment/withdrawal. The untreated SH-SY5Y the epithelioids lack processes (asterisks), neuroblasts show processes (arrows).

Fig. S15. Differential S100 staining of transitional variants (polyclonal Ab to S100A α and β subunits) and MAP-2 + syncytium (mAb M12 to MAP2A/B). Satellites (thick black arrows). Note uneven plasma membrane staining in some transitional variants (blue arrows), vesicles (red arrowheads), outgrowth from satellite (yellow arrow), MAP-2 + fused elongated epithelioids (green arrows). Growth cones (arrowheads).
Fig. S16. MAP-2, Aβ, GFAP, vimentin and APC (mAb CC-1) in satellites (thin arrows) and vesicles (thick arrows). Note that Aβ-processes (arrowheads) outgrow from vesicle.

Fig. S17. S-100, tau and NSE (human brain gamma isoenzyme) in satellites (thin arrows) and vesicles (thick arrows). S-100+ satellite clusters adhered to epithelial (arrows) and transitional forms (arrowheads).
Fig. S18. Tryptamine-induced GFAP+ neuritic swellings (red arrowheads). GFAP+ vesicles (closed arrows), transitional satellite (yellow arrow), epithelioid (green arrow). GFAP(-) and GFAP(+) satellites in control epithelioids (arrows). Note that GFAP(-) (blue arrowheads) and GFAP+(red arrowheads) neurites outgrow from the same epithelioid.

Fig. S19. Synaptophysin (mAb SY38) immunoperoxidase staining after tryptamine treatment withdrawal (25–12 μg/ml tryptamine 35 d, withdrawal 21 d). Multiple synaptophysin+ contacts (black arrowheads), magnified inset shows contacts in cluster of 8 cells. Synaptophysin neurites length is up to 700 μm. Strong synaptophysin+ puncta in neurites (red arrowheads).
Fig. S20. Confocal microscopy of synaptophysin+ growth cones (arrows) and synaptic vesicles in epithelioids with no processes. Epithelioid treatment (50 μM tryptamine 7 d, withdrawal 8 d).

Fig. S21. Interdigitating hybrid junction (a) with the membrane loops (arrowheads) and gaps (arrows). Cell-cell contacts (arrows) between two processes outgrowing in opposite directions (b).
Fig. S22. Intracellular membrane loops. Loops between nuclear membrane and endoplasmic reticulum (area marked). Mitochondrial fusion (M), nuclear and plasma interdigitating membranes (arrowheads).

Fig. S23. Intracellular interdigitating membranes. Interdigitating mitochondrial (black arrows) and nuclear (white arrows) membranes. Plasma membrane-mitochondrial membrane loop (white arrowheads). Rough endoplasmic reticulum (RER), mitochondrial cristolysis (asterisks), fusion of mitochondria (M) with RER. Right images are high magnification of the areas in left image.
Fig. S24. Differential TrpRS staining in cell variants of control SH-SY5Y and after tryptamine treatment/withdrawal (12 μg/ml 5 d, withdrawal 24 d). Control neuroblasts (arrowheads), neuroblastic processes (red arrows), spheroids (purple arrows), vesicles (yellow arrows). After treatment: epithelioid marginal cytoplasmic staining (blue arrows), TrpRS-neurites outgrowing from epithelioids (red arrows), SC typical spindle-shaped body with a prominent nucleus and bipolar extensions (black arrows), TrpRS translocation to periphery (green arrow), axonal swellings (inset, arrowheads).

Fig. S25. Comparison of TrpRS staining in neuronal (a-c) and pancreatic (d) cells. Tryptamine treatment/withdrawal of neuronal cells (12 μg/ml 5 d, withdrawal 24 d). Note predominant TrpRS staining on the satellite membrane (red arrows), MIA PaCa-2 spheroid cytoplasm (d), growth cones and processes (black arrows). Nuclei (N).
**Fig. S26.** TrpRS in alkali-fixed vesicles (pH 10). Tryptamine treatment of epithelioid culture (20 μg/ml 24 d, no withdrawal), extracellular vesicles (blue arrowheads), membrane vesicles (black arrowheads), satellites (red arrows) and no satellite clusters.

**Fig. S27.** TrpRS+ multi-polar neurons resistant to tryptamine (20 μg/ml 24 d, no withdrawal) and alkali fixation (pH 10).
Fig. S28. TrpRS downregulated and translocated to cytoplasmic extensions (black arrows) under hypoxia (0.1% oxygen, 4 h). RT-PCR of TrpRS mRNA. Spheroids (green arrows), nuclei (N).

Fig. S29. Gold TEM of TrpRS in cytoplasmic extension, vesicle partially bound to cells and extracellular vesicles (thick arrows). TrpRS associated with a plasma membrane in the cytoplasmic extension (thin arrows). Organelle (~100 × 50 nm) resembling small mitochondria (arrowhead) located in partially released TrpRS vesicle. Mitochondria (M), fibrils (F), ribosome (R).
Fig. S30. Gold TEM of TrpRS+extracellular vesicles. TrpRS-immunogold product in extracellular space around vesicles (thin arrows). Fused vesicles (thick arrows).

Fig. S31. Gold TEM of TrpRS+growth cone and neurite. TrpRS+extracellular vesicle and plasma membrane vesicle (arrows) adjacent to NFT. Contacts of TrpRS+extracellular vesicles (asterisk) with growth cone.
Fig. S32. High magnification of gold-TEM extracellular electron density associated with vesicles-to-growth cone contacts (double arrows) and TrpRS (arrowheads).

Fig. S33. Gold-TEM of TrpRS in secretory vesicle (arrowheads), mitochondria (M), ribosomes (R) and vacuoles (V). TrpRS extracellular vesicle and cell membrane (arrows).
Fig. S34. Gold-TEM of TrpRS+fibrils (F) in the cytoplasmic extensions/plasma membrane small vesicles (white arrowheads) and extracellular space (black arrowheads).

Fig. S35. Immunoperoxidase TEM of TrpRS+vesicles fixed at pH 10. High magnification insets: vesicles comprise TrpRS+dense deposits and fibrils (F).
Fig. S36. Immunogold electron microscopy of mitochondrial cristolysis in tryptamine-treated epithelial. Note that cytoplasmic TrpRS is detected in mitochondria. TrpRS in association with mitochondrial (M) membranes (arrows) and ribosomes (R); mitochondrial cristolysis (asterisks).

Fig. S37. Transmission electron microscopy micrograph of TrpRS immunogold staining. TrpRS immunoreactivity visualized in association with cytoplasmic extensions (white arrowheads), plasma membrane (black arrows), interdigitating membrane loop (black arrowheads) and ribosomes (R).

Fig. S38. Confocal microscopy. Side views of the TrpRS+ autofluorescent plaque.

Fig. S39. Phase contrast microscopy view of vesicles in the TrpRS+ plaque. Note a compact vesicular core and vesicles around the core.
optical sections | AD hippocampus, TrpRS-plaque

Fig. S40. Confocal laser microscopy. Optical serial sections of the TrpRS+plaque reveal ghost neurons in the plaque.

Fig. S41. TrpRS immunohistochemistry of AD hippocampus with mAb 6C10. TrpRS-extracellular and plasma membrane vesicles (red arrows). TrpRS-neurites (thin black arrows). TrpRS-neuronal plasma membrane (black thick arrow). Note that similar strong TrpRS plasma membrane staining is detected in the tryptamine-treated SH-SYSY-derived progenitor-satellites (Fig. 8C).
Fig. S42. Extracellular vesicles stained in AD, sepsis and acute myocardial infarction brain with anti-TrpRS polyclonal Ab, mAbs 9D7 and 6C10. Numerous TrpRS+extracellular vesicles in AD, fewer in septic brain and no vesicles in myocardial infarction.

Fig. S43. TrpRS immunohistochemistry of epilepsy brain biopsy (hippocampus and temporal lobe) and rheumatism brain autopsy (hippocampus). Epilepsy hippocampus: TrpRS+axon (high magnification inset, arrows), marginal immunostaining of undamaged neurons (red arrows). Neuronal membrane staining (blue arrows), extracellular vesicles (arrowheads). High magnification of TrpRS+neuronal membrane (inset, arrows).
Fig. S44. Double immunostaining of TrpRS (pcAb) and tau (mAb T14) in AD brain. Co-localized immunoreactivity of TrpRS (blue) and tau (brown) in neuronal plasma membrane vesicles (red arrows), extracellular vesicles (yellow arrows) and neuronal plasma membrane fused vesicles (green arrows). Axonal swellings (purple arrowheads). High magnification micrographs of vesicles (insets).

Fig. S45. TrpRS+vesicles and neuronal fragmentation in AD hippocampus. (A) TrpRS+extracellular vesicles in AD hippocampus following formic acid pretreatment of sections (30 min). Immunostaining with anti-TrpRS polyclonal antibody. (B) TrpRS+neuronal membrane vesicles and neuronal fragmentation in AD hippocampus (insets, arrows).
Fig. S46. TrpRS immunohistochemistry (mAb 6C10) of MS hippocampus. TrpRS+ neuronal vesicles (red arrows), extracellular vesicles (black arrowheads), axons (green arrows), TrpRS+ vesicular plaque (inset). The control staining of brain section of the same MS case with non-secretory hybridoma is negative.

Fig. S47. TrpRS and Aβ colocalization in plasma membrane and axonal membrane vesicles (black arrowheads) of neurons (a, b) with mitotic figures (blue arrowheads) in AD hippocampus.
Fig. S48. Confocal laser microscopy of hippocampal dividing neurons (a, b) in AD brain. Light microscopy of TrpRS immunoperoxidase staining of these neurons as insets.
Fig. S49. TrpRS immunostaining of mouse brain sections. TrpRS immunoreactivity (pcAb) associated with occasional vesicles (arrow) at the neuronal plasma membrane (inset). Control mouse was injected with PBS. TrpRS-mouse was immunized with TrpRS. No TrpRS accumulation observed in hippocampus of mouse immunized with TrpRS. Immunostaining with secondary antibodies is negative.