Supplemental Materials

Molecular Biology of the Cell

Gan et al.

Table S1: Quantitative analysis of dendritic and axonal DCV transport in tau $^{+/+}$ and tau $^{-/-}$ neurons

	Dense Core Vesicles						%	
	All Events		Anterograde		Retrograde		All Events	
	Dendrites	Axons	Dendrites	Axons	Dendrites	Axons	Dendrites	Axons
Flux (min $^{-1}$)								
tau $^{+/+}$ vehicle	8.96 ± 0.45	18.62 ± 2.06	4.61 ± 0.29	9.81 ± 1.42	4.35 ± 0.23	8.81 ± 0.95	100.00 ± 5.02	100.00 ± 11.06
tau $^{+/+}$ AβOs 13-18 h	4.89 ± 0.44***	14.61 ± 1.93*	2.58 ± 0.23***	7.36 ±1.49*	2.31 ± 0.25***	7.24 ± 0.75*	54.58 ± 4.91***	78.46 ± 10.37*
tau $^{+/+}$ AβOs + FK506	7.23 ± 0.39$^{**/\#\#\#}$	--	3.84 ± 0.24$^{*/\#\#\#}$	--	3.39 ± 0.24$^{**/\#\#\#}$	--	80.69 ± 4.35$^{**/\#\#\#}$	--
tau $^{-/-}$ vehicle	7.66 ± 0.68	17.76 ± 1.25	3.75 ± 0.35	9.38 ± 0.82	3.91 ± 0.36	8.66 ± 0.67	85.49 ± 7.59	95.38 ± 6.71
tau $^{-/-}$ AβOs 13-18 h	3.41 ± 0.27***	14.38 ± 1.41	1.78 ± 0.15***	7.59 ± 0.87	1.64 ± 0.13***	6.79 ± 0.65	38.06 ± 3.01***	77.23 ± 7.57
tau $^{-/-}$ AβOs + FK506	7.56 ± 0.65$^{\#\#\#}$	--	3.84 ± 0.34$^{\#\#\#}$	--	3.72 ± 0.33$^{\#\#\#}$	--	84.38 ± 7.25$^{\#\#\#}$	--
Velocity (µm/s)								
tau $^{+/+}$ vehicle	1.43 ± 0.03	1.86 ± 0.12	1.44 ± 0.04	1.94 ± 0.12	1.42 ± 0.04	1.78 ± 0.13	100.00 ± 2.10	100.00 ± 6.45
tau $^{+/+}$ AβOs 13-18 h	1.34 ± 0.03*	1.85 ± 0.12	1.30 ± 0.04**	1.88 ± 0.14	1.37 ± 0.04	1.82 ± 0.13	93.71 ± 2.10*	99.46 ± 6.45
tau $^{+/+}$ AβOs + FK506	1.27 ± 0.04**	--	1.22 ± 0.05***	--	1.32 ± 0.04	--	88.81 ± 2.80**	--
tau $^{-/-}$ vehicle	1.31 ± 0.05*	2.03 ± 0.08	1.30 ± 0.06*	2.06 ± 0.09	1.33 ± 0.04	1.99 ± 0.08	91.61 ± 3.50*	109.14 ± 4.30
tau $^{-/-}$ AβOs 13-18 h	1.19 ± 0.03	2.10 ± 0.09	1.16 ± 0.04	2.13 ± 0.08	1.23 ± 0.04	2.07 ± 0.10	83.22 ± 2.10	112.90 ± 4.84
tau $^{-/-}$ AβOs + FK506	1.35 ± 0.04$^{\#\#}$	--	1.36 ± 0.04$^{\#\#}$	--	1.35 ± 0.04$^{\#}$	--	94.41 ± 2.80$^{\#\#}$	--
Run length (µm)								
tau $^{+/+}$ vehicle	4.53 ± 0.25	8.60 ± 1.14	4.44 ± 0.26	9.28 ± 1.32	4.68 ± 0.27	7.73 ± 0.88	100.00 ± 5.52	100.00 ± 13.26
tau $^{+/+}$ AβOs 13-18 h	4.56 ± 0.17	8.83 ± 0.82	4.54 ± 0.21	9.49 ± 1.25	4.64 ± 0.23	8.26 ± 0.95	100.66 ± 3.75	102.67 ± 9.29
tau $^{+/+}$ AβOs + FK506	5.06 ± 0.16$^{\#}$	--	5.01 ± 0.22	--	5.19 ± 0.17	--	111.70 ± 3.53$^{\#}$	--
tau $^{-/-}$ vehicle	5.11 ± 0.16	10.36 ± 0.46	4.92 ± 0.22	11.24 ± 0.55*	5.67 ± 0.16	9.80 ± 0.43**	112.80 ± 3.53	120.47 ± 4.44
tau $^{-/-}$ AβOs 13-18 h	4.75 ± 0.14	10.34 ± 0.09	4.77 ± 0.18	11.45 ± 0.08	4.79 ± 0.16	9.45 ± 0.64	104.86 ± 3.09	120.23 ± 0.87
tau $^{-/-}$ AβOs + FK506	5.48 ± 0.15$^{\#\#}$	--	5.50 ± 0.08$^{\#}$	--	5.54 ± 0.18$^{\#\#}$	--	120.97 ± 3.31$^{\#\#}$	--

tau$^{+/+}$ vehicle dendrites: n=18 kymographs (18 cells, 1187 vesicles); axons: n=15 kymographs (15 cells, 1753 vesicles)
tau$^{+/+}$ AβO dendrites: n=22 kymographs (22 cells, 710 vesicles); axons: n=16 kymographs (16 cells, 1383 vesicles)
tau$^{+/+}$ AβO + FK506 dendrites: n=22 kymographs (22 cells, 919 vesicles)
tau$^{-/-}$ vehicle dendrites: n=17 kymographs (17 cells, 739 vesicles); axons: n=15 kymographs (15 cells, 1423 vesicles)
tau$^{-/-}$ AβO dendrites: n=34 kymographs (34 cells, 737 vesicles); axons: n=18 kymographs (18 cells, 1276 vesicles)
tau$^{-/-}$ AβO + FK506 dendrites: n=21 kymographs (21 cells, 909 vesicles)

* p<0.05, when compared with vehicle
**p<0.01, when compared with vehicle
*** p<0.001, when compared with vehicle

# p<0.05, when compared with AβOs
p<0.01, when compared with AβOs
p<0.001, when compared with AβOs

Table S2: VGCC inhibition prevents AβO-induced transport defects in tau $^{+/+}$ and tau $^{-/-}$ neurons

	Dense Core Vesicles						%	
	All Events		Anterograde		Retrograde		All Events	
	Dendrites	Axons	Dendrites	Axons	Dendrites	Axons	Dendrites	Axons
Flux (min $^{-1}$)								
tau $^{+/+}$ vehicle	8.96 ± 0.45	16.79 ± 1.44	4.61 ± 0.29	9.99 ± 0.68	4.35 ± 0.23	6.80 ± 0.94	100.00 ± 5.02	100.00 ± 4.99
tau $^{+/+}$ AβOs	4.89 ± 0.44***	8.04 ± 0.98***	2.58 ± 0.23***	5.08 ±0.62***	2.31 ± 0.25***	2.96 ± 0.40***	54.58 ± 4.99***	47.84 ± 5.82***
tau $^{+/+}$ Aga + AβOs	3.94 ± 0.39***	14.91 ± 0.84^{+++}	2.29 ± 0.24***	8.62 ± 0.56^{+++}	1.68 ± 0.03***	6.28 ±0.48^{+++}	43.96 ± 3.88***	88.76 ± 4.99^{+++}
tau $^{+/+}$ Cono + AβOs	4.11 ± 0.33***	16.58 ± 0.83^{+++}	2.61 ± 0.27***	10.11 ± 0.49^{+++}	1.50 ± 0.21***	6.46 ± 0.61^{+++}	45.92 ± 3.76***	98.72 ± 5.00^{+++}
tau $^{+/+}$ Nimo + AβOs	3.83 ± 0.23***	7.96 ± 0.96***	2.06 ± 0.15***	4.95 ± 0.75***	1.76 ± 0.13***	3.00 ± 0.52***	42.76 ± 2.60***	47.39 ± 5.74***
tau $^{+/+}$ EGTA + AβOs	--	15.30 ± 0.97^{+++}	--	9.87 ± 0.61^{+++}	--	5.43 ± 0.57^{+++}	--	91.14 ± 5.82^{+++}
tau $^{-/-}$ vehicle	7.66 ± 0.68	17.47 ± 0.79	3.75 ± 0.35	9.39 ± 0.59	3.91 ± 0.36	8.07 ± 0.59	100.49 ± 7.12	100.00 ± 4.56
tau $^{-/-}$ AβOs	3.41 ± 0.27***	6.72 ± 0.55***	1.78 ± 0.15***	4.40 ± 0.39***	1.64 ± 0.13***	2.32 ± 0.26***	45.04 ± 4.03***	38.51 ± 3.19***
tau $^{-/-}$ Aga + AβOs	4.24 ± 0.48***	15.62 ± 0.59^{+++}	2.58 ± 0.33***	9.07 ± 0.62^{+++}	1.98 ± 0.07***	6.54 ± 0.45^{+++}	58.96 ± 6.80***	83.25 ± 5.90^{+++}
tau $^{-/-}$ Cono + AβOs	4.45 ± 0.50***	14.5 ± 1.03^{+++}	2.38 ± 0.29***	8.82 ± 0.55^{+++}	2.16 ± 0.21***	5.72 ± 0.58^{+++}	61.84 ± 6.99***	89.40 ± 3.35^{+++}
tau $^{-/-}$ Nimo + AβOs	3.53 ± 0.28***	6.94 ± 0.56***	1.79 ± 0.16***	4.76 ± 0.41***	1.74 ± 0.12***	2.17 ± 0.56***	49.12 ± 3.89***	39.73 ± 3.22***
tau $^{-/-}$ EGTA + AβOs	--	14.6 ± 0.92^{+++}	--	8.87 ± 0.60^{+++}	--	6.40 ± 0.54^{+++}	--	83.63 ± 5.27^{+++}
Velocity (µm/s)								
tau $^{+/+}$ vehicle	1.60 ± 0.03	1.60 ± 0.08	1.44 ± 0.04	1.72 ± 0.07	1.42 ± 0.04	1.43 ± 0.10	100.00 ± 2.10	100.00 ± 5.07
tau $^{+/+}$ AβOs	1.34 ± 0.03*	1.32 ± 0.05**	1.30 ± 0.04**	1.40 ± 0.06**	1.37 ± 0.08	1.21 ± 0.05**	93.71 ± 2.34	82.33 ± 3.35**
tau $^{+/+}$ Aga + AβOs	1.52 ± 0.02	1.62 ± 0.07^{++}	1.58 ± 0.03	1.67 ± 0.06^{++}	1.46 ± 0.06	1.39 ± 0.08^{++}	95.31 ± 3.10	101.11 ± 3.60^{++}
tau $^{+/+}$ Cono + AβOs	1.61 ± 0.07	1.74 ± 0.08^{++}	1.65 ± 0.05	1.92 ± 0.11^{++}	1.58 ± 0.03	1.58 ± 0.07^{++}	100.01 ± 2.80	108.71 ± 5.25^{++}
tau $^{+/+}$ Nimo + AβOs	1.22 ± 0.10	1.16 ± 0.08**	1.27 ± 0.07	1.24 ± 0.08**	1.17 ± 0.01	1.06 ± 0.08**	76.21 ± 2.15	72.74 ± 5.00 **
tau $^{+/+}$ EGTA + AβOs	--	1.65 ± 0.07^{++}	--	1.77 ± 0.08^{++}	--	1.50 ± 0.07^{++}	--	103.56 ± 4.91^{++}
tau $^{-/-}$ vehicle	1.31 ± 0.05	1.68 ± 0.06	1.30 ± 0.06	1.78 ± 0.07	1.33 ± 0.01	1.60 ± 0.08	100.00 ± 3.50	100.00 ± 3.85
tau $^{-/-}$ AβOs	1.19 ± 0.03	1.12 ± 0.04**	1.24 ± 0.04	1.20 ± 0.08**	1.17 ± 0.07	1.02 ± 0.05**	90.22 ± 2.10	66.68 ± 2.78**
tau $^{-/-}$ Aga + AβOs	1.48 ± 0.05	1.63 ± 0.05^{++}	1.50 ± 0.03	1.73 ± 0.02^{++}	1.46 ± 0.06	1.53 ± 0.07^{++}	102.41 ± 2.31	97.46 ± 3.23^{++}
tau $^{-/-}$ Cono + AβOs	1.34 ± 0.09	1.48 ± 0.05^{++}	1.36 ± 0.01	1.55 ± 0.07^{++}	1.32 ± 0.04	1.40 ± 0.07^{++}	102.96 ± 3.75	88.47 ± 3.22^{++}
tau $^{-/-}$ Nimo + AβOs	1.35 ± 0.08	1.21 ± 0.05**	1.36 ± 0.09	1.34 ± 0.06**	1.35 ± 0.03	1.05 ± 0.06**	94.41 ± 0.32	72.11 ± 3.53**
tau $^{-/-}$ EGTA + AβOs	--	1.58 ± 0.06^{++}	--	1.67 ± 0.06^{++}	--	1.49 ± 0.07^{++}	--	93.91 ± 3.78^{++}
Run length (µm)								
tau $^{+/+}$ vehicle	4.53 ± 0.25	15.54 ± 0.80	4.44 ± 0.26	18.63 ± 1.38	4.68 ± 0.27	12.19 ± 1.04	100.00 ± 5.52	100.00 ± 5.17
tau $^{+/+}$ AβOs	4.56 ± 0.17	10.19 ± 0.39**	4.54 ± 0.21	12.42 ± 0.63**	4.64 ± 0.23	7.61 ± 0.16**	100.66 ± 3.75	65.59 ± 2.55**
tau $^{+/+}$ Aga + AβOs	5.23 ± 0.16	12.18 ± 0.51	5.21 ± 0.27	14.91 ± 0.87	5.24 ± 0.17	9.49 ± 0.41	115.45± 3.53	78.36 ± 3.31
tau $^{+/+}$ Cono + AβOs	5.17 ± 0.20	13.60 ± 0.83	5.22 ± 0.29	17.45 ± 0.48	5.15 ± 0.18	10.66 ± 0.61	114.70 ± 4.62	87.49 ± 5.40

tau $^{+/+}$ Nimo + AβOs	5.06 ± 0.14	11.09 ± 0.90	5.10 ± 0.19	12.50 ± 1.11	5.02 ± 0.24	9.04 ± 1.01	111.69± 4.58	71.38 ± 5.78
tau $^{+/+}$ EGTA + AβOs	4.27 ± 0.10	15.87 ± 0.55^{++}	4.34 ± 0.30	19.29 ± 0.77^{++}	4.20 ± 0.19	11.29 ± 0.45^{++}	94.26 ± 3.72	102.11 ± 3.54^{++}
tau $^{-/-}$ vehicle	5.11 ± 0.16	13.75 ± 0.56	5.17 ± 0.28	17.19 ± 1.03	5.08 ± 0.26	13.75 ± 0.56	100.00 ± 3.59	100.00 ± 4.07
tau $^{-/-}$ AβOs	4.75 ± 0.14	11.31 ± 0.52	4.77 ± 0.31	13.95 ± 0.86	4.72 ± 0.13	8.31 ± 0.36	92.95 ± 3.09	82.30 ± 3.83
tau $^{-/-}$ Aga + AβOs	5.48 ± 0.15	14.09 ± 0.53	5.50 ± 0.26	17.2 ± 0.91	5.45 ± 0.31	11.06 ± 0.45	107.25 ± 3.31	102.39 ± 3.82
tau $^{-/-}$ Cono + AβOs	4.99 ± 0.12	13.89 ± 0.42	5.50 ± 0.29	16.94 ± 0.71	4.73 ± 0.28	10.87 ± 0.43	97.67 ± 2.99	101.03 ± 3.73
tau $^{-/-}$ Nimo + AβOs	5.23 ± 0.18	13.00 ± 0.74	5.30 ± 0.21	15.98 ± 0.93	5.17 ± 0.25	9.42 ± 0.90	102.34 ± 3.13	94.52 ± 5.37
tau $^{-/-}$ EGTA + AβOs	--	13.99 ± 0.51	--	17.78 ± 0.87	--	11.11 ± 0.53	--	101.75 ± 3.73

---

tau$^{+/+}$ vehicle dendrites: n=18 kymographs (18 cells, 1187 vesicles); axons: n=15 kymographs (15 cells, 886 vesicles)
tau$^{+/+}$ AβO dendrites: n=22 kymographs (22 cells, 710 vesicles); axons: n=16 kymographs (16 cells, 544 vesicles)
tau$^{+/+}$ Agatoxin + AβO dendrites: n=15 kymographs (15 cells, 510 vesicles); axons: n=18 kymographs (18 cells, 1037 vesicles)
tau$^{+/+}$ Conotoxin + AβO dendrites: n=15 kymographs (15 cells, 550 vesicles); axons: n=17 kymographs (17 cells, 893 vesicles)
tau$^{+/+}$ Nimodipine + AβO dendrites: n=15 kymographs (15 cells, 268 vesicles); axons: n=15 kymographs (15 cells, 538 vesicles)
tau$^{+/+}$ EGTA + AβO axons: n=15 kymographs (15 cells, 695 vesicles)

tau$^{-/-}$ vehicle dendrites: n=17 kymographs (17 cells, 718 vesicles); axons: n=15 kymographs (15 cells, 943 vesicles)
tau$^{-/-}$ AβO dendrites: n=28 kymographs (28 cells, 589 vesicles); axons: n=18 kymographs (18 cells, 534 vesicles)
tau$^{-/-}$ Agatoxin + AβO dendrites: n=15 kymographs (15 cells, 536 vesicles); axons: n=16 kymographs (16 cells, 728 vesicles)
tau$^{-/-}$ Conotoxin + AβO dendrites: n=15 kymographs (15 cells, 475 vesicles); axons: n=16 kymographs (16 cells, 723 vesicles)
tau$^{-/-}$ Nimodipine + AβO dendrites: n=15 kymographs (15 cells, 503 vesicles); axons: n=16 kymographs (16 cells, 402 vesicles)
tau$^{-/-}$ EGTA + AβO axons: n=16 kymographs (16 cells, 711 vesicles)

* $p<0.05$, when compared with vehicle
**$p<0.01$, when compared with vehicle
*** $p<0.001$, when compared with vehicle

+ $p<0.05$, when compared with AβOs
++ $p<0.01$, when compared with AβOs
+++ $p<0.001$, when compared with AβOs

Table S3: RyR inhibition prevents AβO-induced transport defects in tau$^{+/+}$and tau$^{-/-}$ neurons

	Dense core vesicles			
	Traffic values			%
	All events	Anterograde	Retrograde	All events (%)
Flux (min^{-1})				
tau $^{+/+}$ vehicle	15.97 ± 1.20	9.37 ± 0.82	6.61 ± 0.69	100.00 ± 6.56
tau $^{+/+}$ AβOs	7.85 ± 1.64***	5.52 ± 1.18***	2.33 ± 0.59***	47.89 ± 6.92***
tau $^{+/+}$ dantrolene + AβOs	14.79 ± 1.39^{+++}	9.25 ± 0.72^{+++}	5.54 ± 0.97^{+++}	97.47 ± 10.37^{+++}
tau $^{-/-}$ vehicle	17.32 ± 1.89	11.25 ± 1.19	6.06 ± 0.99	100.00 ± 6.77
tau $^{-/-}$ AβOs	7.68 ± 0.83***	4.52 ± 0.75***	3.15 ± 0.35***	33.35 ± 5.30***
tau $^{-/-}$ dantrolene + AβOs	16.83 ± 2.14^{+++}	10.35 ± 1.42^{+++}	6.48 ± 0.96^{+++}	96.06 ± 7.92^{+++}
Velocity (µm/s)				
tau $^{+/+}$ vehicle	1.39 ± 0.05	1.51± 0.06	1.28 ± 0.04	100.00 ± 4.12
tau $^{+/+}$ AβOs	1.17 ± 0.05*	1.44 ± 0.07*	1.05 ± 0.04**	72.51 ± 4.85*
tau $^{+/+}$ dantrolene + AβOs	1.37 ± 0.08^{+}	1.49 ± 0.09^{+}	1.26 ± 0.09^{++}	95.85 ± 3.43^{+}
tau $^{-/-}$ vehicle	1.50 ± 0.12	1.65 ± 0.11	1.33 ± 0.10	100.00 ± 5.76
tau $^{-/-}$ AβOs	1.09 ± 0.11***	1.14 ± 0.12**	1.01 ± 0.13***	67.58 ± 3.98***
tau $^{-/-}$ dantrolene + AβOs	1.44 ± 0.09^{+++}	1.53 ± 0.09^{++}	1.32 ± 0.10^{+++}	97.32 ± 5.81^{+++}
Run length (µm)				
tau $^{+/+}$ vehicle	9.29 ± 0.48	11.12 ± 0.32	7.14 ± 0.34	100.00 ± 8.37
tau $^{+/+}$ AβOs	9.63 ± 0.94	11.41 ± 1.15	6.41 ± 0.46	103.98 ± 2.90
tau $^{+/+}$ dantrolene + AβOs	9.42 ± 0.94	10.62 ± 0.83	6.69 ± 0.90	101.64 ± 8.48
tau $^{-/-}$ vehicle	12.34 ± 0.72	15.39 ± 0.83	8.89 ± 0.73	100.00 ± 5.75
tau $^{-/-}$ AβOs	8.36 ± 0.78**	8.52 ± 1.05**	7.96 ± 1.43**	74.40 ± 4.76**
tau $^{-/-}$ dantrolene + AβOs	10.22 ± 0.79^{++}	12.08 ± 0.92^{++}	8.19 ± 0.85^{+}	83.67 ± 7.50^{++}

tau$^{+/+}$ vehicle: n=15 kymographs (15 cells, 1261 vesicles)
tau$^{+/+}$ AβO: n=15 kymographs (15cells, 1005 vesicles)
tau$^{+/+}$ dantrolene + AβO: n=15 kymographs (15 cells, 1097 vesicles)
tau$^{-/-}$ vehicle: n=15 kymographs (17 cells, 2167 vesicles)
tau$^{-/-}$ AβO: n=15 kymographs (19 cells, 933 vesicles)
tau$^{-/-}$ dantrolene + AβO: n=15kymographs (16 cells, 1666 vesicles)

* p<0.05, when compared with vehicle
** p<0.01, when compared with vehicle
*** p<0.001, when compared with vehicle
+ p<0.05, when compared with AβOs
++ p<0.01, when compared with AβOs
+++ p<0.001, when compared with AβOs

SUPPLEMENTAL MOVIE LEGENDS

Movie S1: Dendritic transport of BDNF-RFP in cultured hippocampal neurons. Live imaging showing dendritic BDNF transport in control versus AβO-treated (18h, 500 nM) neurons. Note the significant difference between BDNF-mRFP vesicle flux in controls compared to AβO-treated neurons. When neurons exposed to 500 nM AβOs are subsequently treated with 1μM FK506, a calcineurin inhibitor, dendritic transport of BDNF-mRFP is rescued. 4 frames/second for 30 seconds; Scale = 10 μm.

Movie S2: Axonal transport of BDNF-RFP in cultured hippocampal neurons. Live imaging showing BDNF transport in control versus AβO-treated (18h, 500 nM) neurons. Note the significant difference between BDNF-mRFP vesicle flux in controls compared to AβO-treated neurons. When neurons are pretreated with 0.5 μM dantrolene and then exposed to 500 nM AβOs, BDNF-mRFP transport defects are prevented. 4 frames/second for 30 seconds; Scale = 5 μm.