

Figure 3. PrP-induced fast axonal transport inhibition is mediated by Casein kinase 2 activity.

Data from **Fig. 3 (A-D)** corresponding to plots that depict vesicle motility in extruded axoplasms, and graphs (**C** and **D**) showing quantitation of average rates of anterograde and retrograde fast axonal transport obtained 30-50 minutes after PrP perfusion from three independent experiments:

Data from plots that depict the vesicle velocity in extruded axoplasms measured in $\mu\text{m}/\text{sec}$:

Treatment	Anterograde	Retrograde
PrP 106-126 + DMAT	1,621	1,233
PrP 106-126 + DMAT	1,524	1,231
PrP 106-126 + DMAT	1,679	1,207
Prion-FL + DMAT	1,679	1,249
Prion-FL + DMAT	1,46	1,14
Prion-FL + DMAT	1,58	1,167

Data from graph (**E**) corresponding to CK2 activity evaluated *in vitro* by CK2 kinase assay. Phosphorylation kinase activity of CK2 is expressed as the incorporation of radioactive inorganic phosphate (P_i) into the synthetic $R_3A_2DSD_5$ peptide by the recombinant CK2. The data suggest that PrP activates CK2 directly. Values are expressed as C.P.M., which stands for counts per minute in arbitrary units. Scintillation counting-based quantitation from 3 independent experiments. *: $p < 0.0002$; **: $p < 0.0001$. Two-tailed P values:

CK2+Sub.	CK2+Sub+DMAT	PrP-FL	PrP ₁₀₆₋₁₂₆
28545,9	13304,9	90155,5	64771,5
29077,9	15516,4	89876,1	64536,0
29030,7	11399,3	89638,8	62138,1

Data from figure 3 (**H** and **I**) corresponding to kymograms (**F** and **G**), which depict the mean mitochondria traveled distance in the anterograde or retrograde direction (**H**) and the percentage of moving mitochondria (**I**) upon PrP or PrP plus the specific CK2 inhibitor DMAT treatment. Quantitative analysis confirmed that average distances traveled by individual mitochondria in either anterograde ($6.56 \pm 2.09 \mu\text{m}$) or retrograde ($11.05 \pm 2.74 \mu\text{m}$) direction were significantly higher in neurons co-treated with DMAT compared to average distances of individual mitochondria from PrP₁₀₆₋₁₂₆ treated neurons (anterograde: $1.21 \pm 0.38 \mu\text{m}$; retrograde $3.97 \pm 1.44 \mu\text{m}$) (**H**). Additionally, the percentage of moving mitochondria (**I**) was significantly higher in DMAT-treated neurons ($45.51 \pm 7.29 \mu\text{m}$) than in PrP₁₀₆₋₁₂₆ treated neurons ($14.72 \pm 8.17 \mu\text{m}$). Collectively, results from these experiments indicated that the inhibitory effects of PrP-FL and PrP₁₀₆₋₁₂₆ on FAT are mediated by CK2 endogenous activity. Results were obtained from 3 independent experiments. One-way ANOVA with post-hoc Tukey.

Treatment	Direction	Experiment	# Neurons	# Mitochondria	Moving Distance (μm)
PrP106-126	Anterograde	1	5	3	0.56
PrP106-126	Anterograde	2	3	2	1.48
PrP106-126	Anterograde	3	4	2	1.62
PrP106-126	Retrograde	1	3	10	1.13
PrP106-126	Retrograde	2	2	4	5.86
PrP106-126	Retrograde	3	2	7	4.91
PrP106-126+DMAT	Anterograde	1	4	6	10.49
PrP106-126+DMAT	Anterograde	2	3	3	3.29
PrP106-126+DMAT	Anterograde	3	4	4	5.89
PrP106-126+DMAT	Retrograde	1	4	11	6.03
PrP106-126+DMAT	Retrograde	2	3	5	11.61
PrP106-126+DMAT	Retrograde	3	4	9	15.50