

Examining an endogenous modulator of chaperone-mediated autophagy across Braak stages in Alzheimer’s disease

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Background

Defective clearance of tau by neuronal autophagy has been implicated in the accumulation of toxic forms of tau in Alzheimer’s disease (AD; Martinez Vicente & Cuervo, 2007). A novel, endogenous activator of chaperone-mediated autophagy (CMA-modulator) was identified in cell culture experiments (Fig. 1; Cuervo laboratory, unpublished data). This CMA-modulator has not been previously studied in AD. Hypothesis: loss of CMA-modulator in neurons leads to decreased clearance of abnormal tau monomers, promoting tau accumulation.

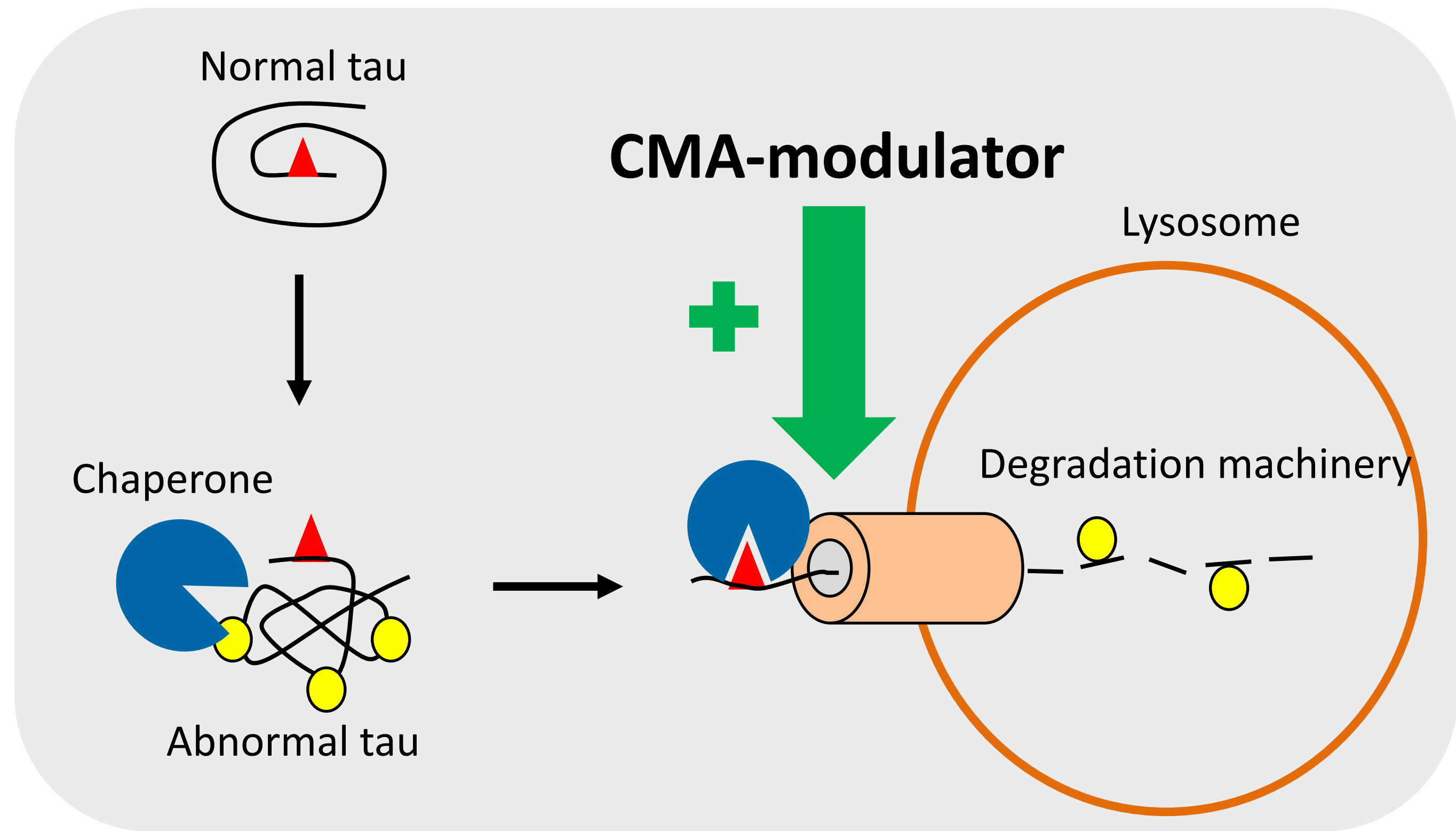


Figure 1. A novel, endogenous modulator of chaperone-mediated autophagy (CMA-modulator) activates degradation of abnormal tau monomers.

Methods

Multiplex immunofluorescence (CMA-modulator, NeuN for neurons, IF3C for acetyl-tau, and AT100 for phospho-tau) was performed on tissue microarrays in the inferior temporal gyrus of 17 AD cases and controls (Table 1; Ehrenberg et al, 2020; Caballero et al, 2021). For each subject, marker expression was quantified within a gray matter area of 7.4 - 7.6 mm² using digital pathology (Figs. 2, 3). To analyze the change in percentage of neurons positive for CMA-modulator across Braak stages, we fit a linear regression model (Fig. 4).

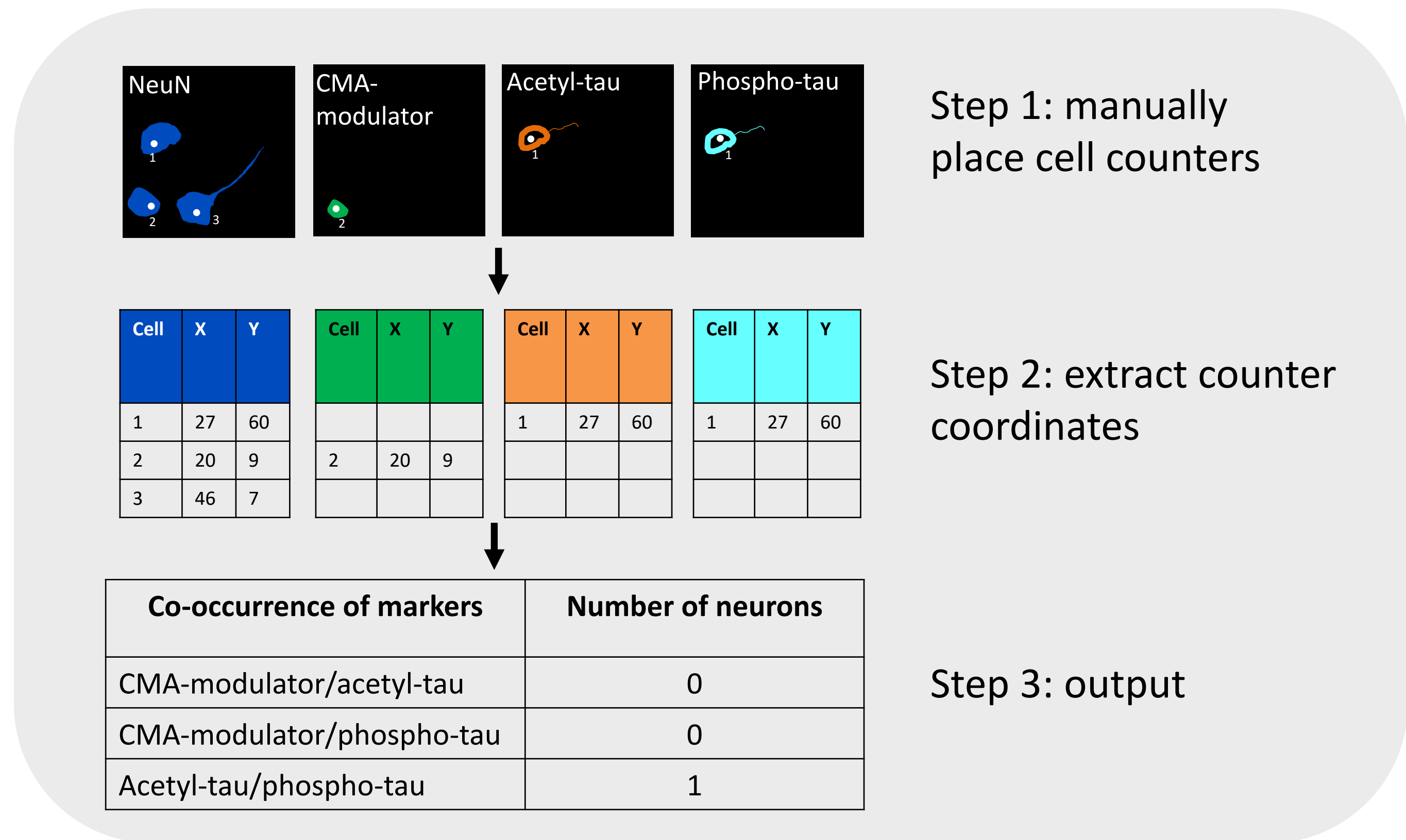


Figure 2. Schematic of cell counting method.

Results

In amnesic AD, the percentage of neurons positive for CMA-modulator declined linearly with advancing Braak stage, but this trend was not statistically significant (Fig. 4; slope = 2.42% loss per Braak stage, $p = 0.086$). There was no significant association between the percentage of CMA-modulator positive neurons and either age or gender. Quantification of the acetyl-tau and phospho-tau markers is ongoing.

Table 1. Subject characteristics. CBS = corticobasal syndrome. lvPPA = logopenic variant of primary progressive aphasia, fvAD = frontal variant AD, and PCA = posterior cortical atrophy.

Case #	Clinical Diagnosis	Braak stage	Sex	Age	Number of neurons (NeuN)	Number of CMA-modulator+ neurons	Percent of neurons CMA-modulator+
01	control	0	F	60	2022	495	24.5
02	control	1	F	82	2181	326	15.0
03	control	2	M	76	2308	727	31.5
04	control	2	M	91	1569	224	14.3
05	amnesic	3	F	88	2383	86	3.6
06	amnesic	3	M	89	1855	192	10.3
07	amnesic	4	M	91	2167	596	27.5
08	control	4	M	103	2647	373	14.1
09	amnesic	5	M	73	2574	275	10.7
10	control	5	M	77	1838	36	2.0
11	amnesic	5	M	81	2887	491	17.0
12	amnesic	6	M	64	2474	46	1.9
13	amnesic	6	F	58	3290	414	12.6
14	CBS	6	F	69	2302	782	34.0
15	lvPPA	6	F	75	977	753	77.1
16	fvAD	6	M	69	2057	4	0.2
17	PCA	6	F	64	2159	16	0.7

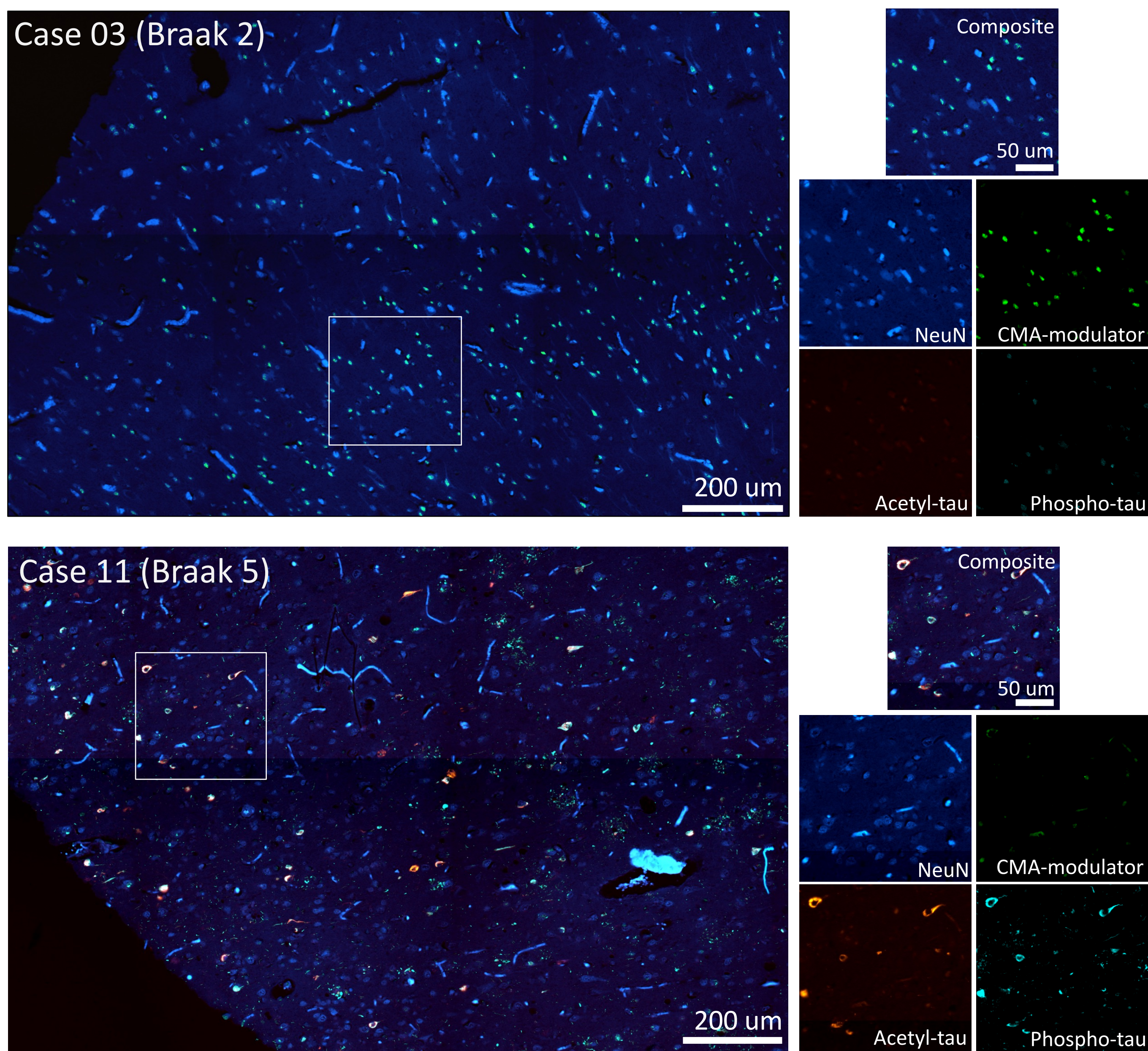


Figure 3. Examples of marker positivity in the inferior temporal gyrus.

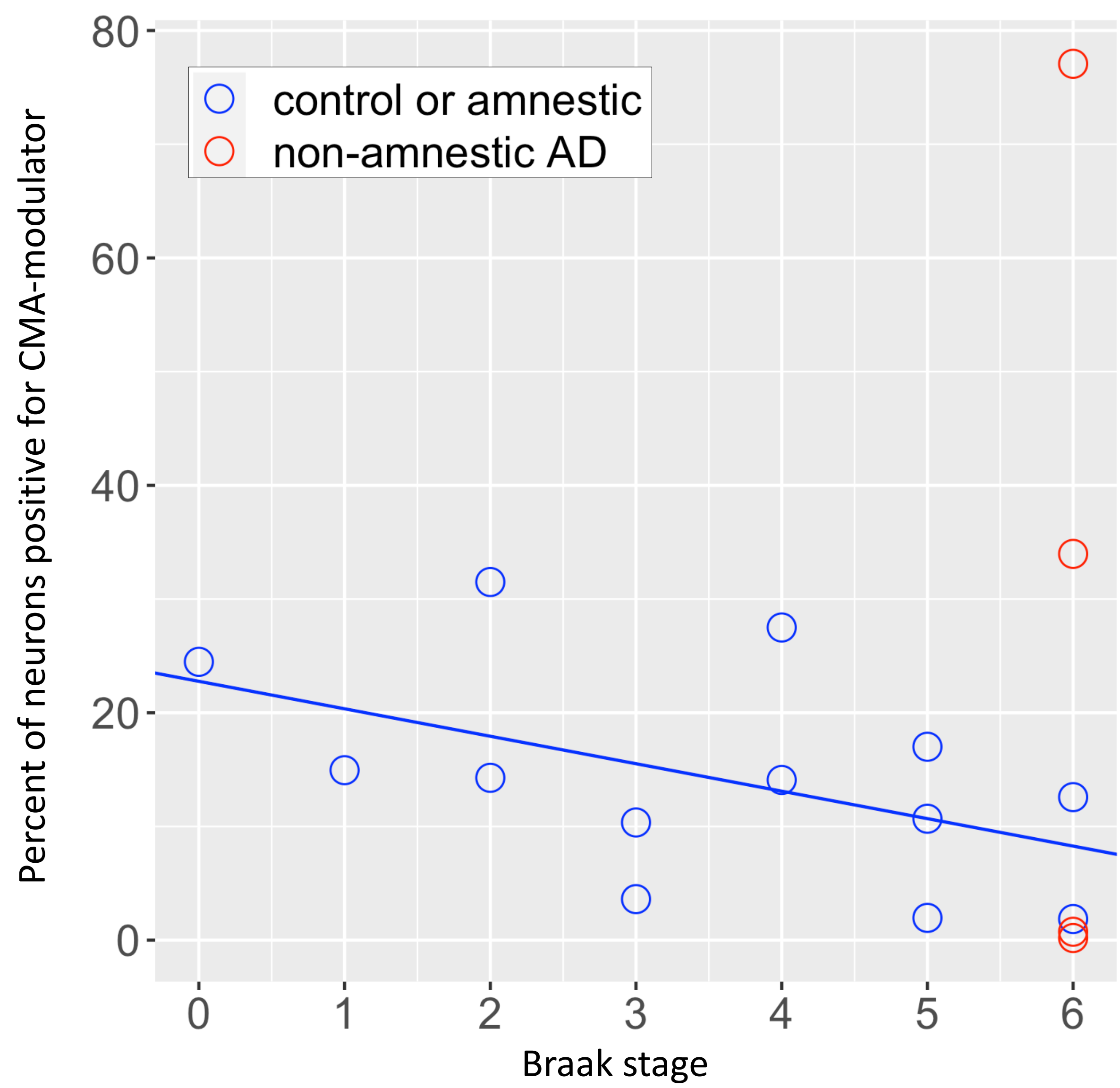


Figure 4. Percent of neurons positive for CMA-modulator in the inferior temporal gyrus plotted against Braak stage.

Conclusions

There may be a gradual loss of the endogenous CMA-modulator across Braak stages in amnesic AD, but this study was underpowered to draw definitive conclusions. We are increasing the sample size and troubleshooting our immunofluorescence protocol.

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