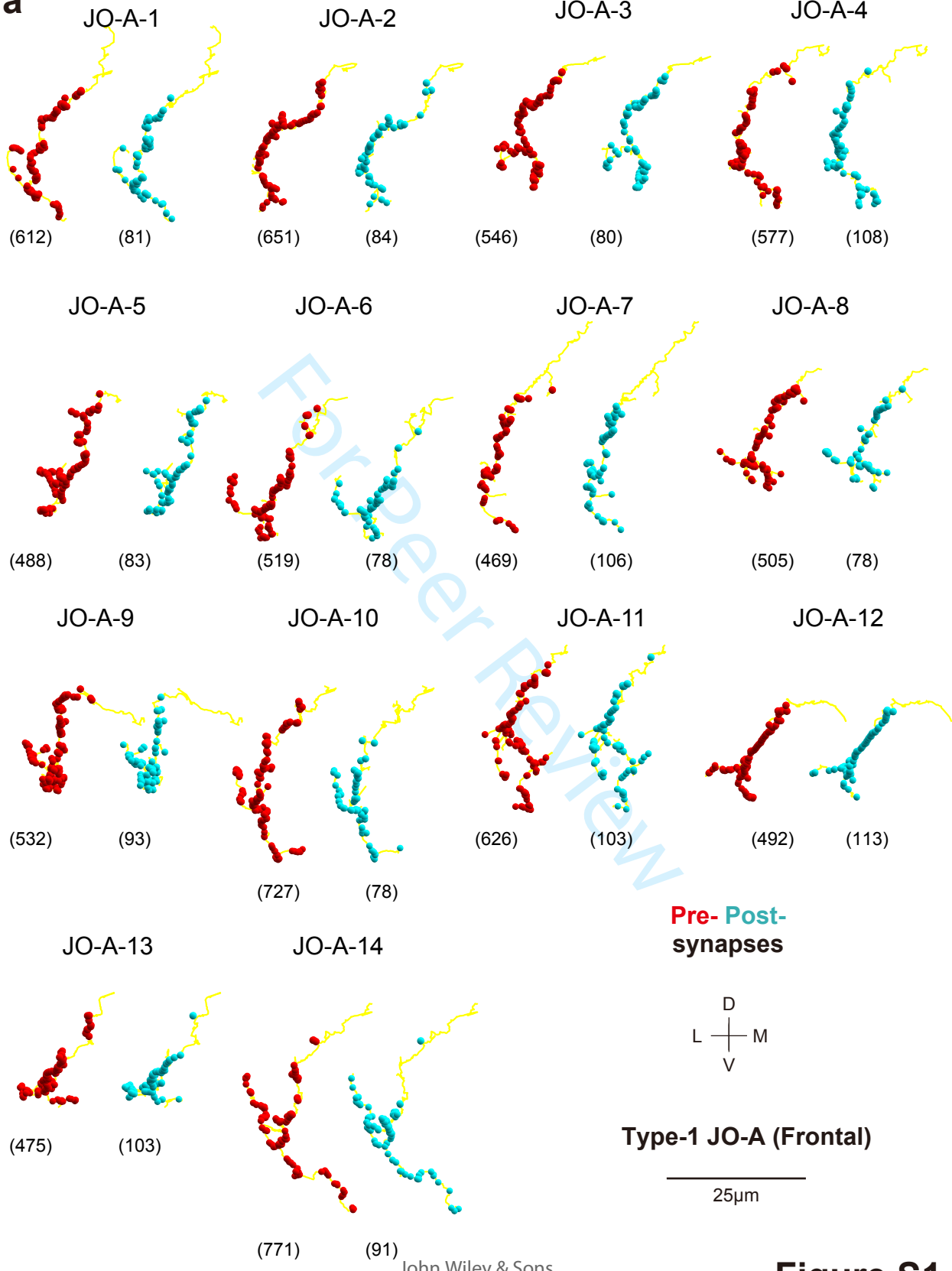
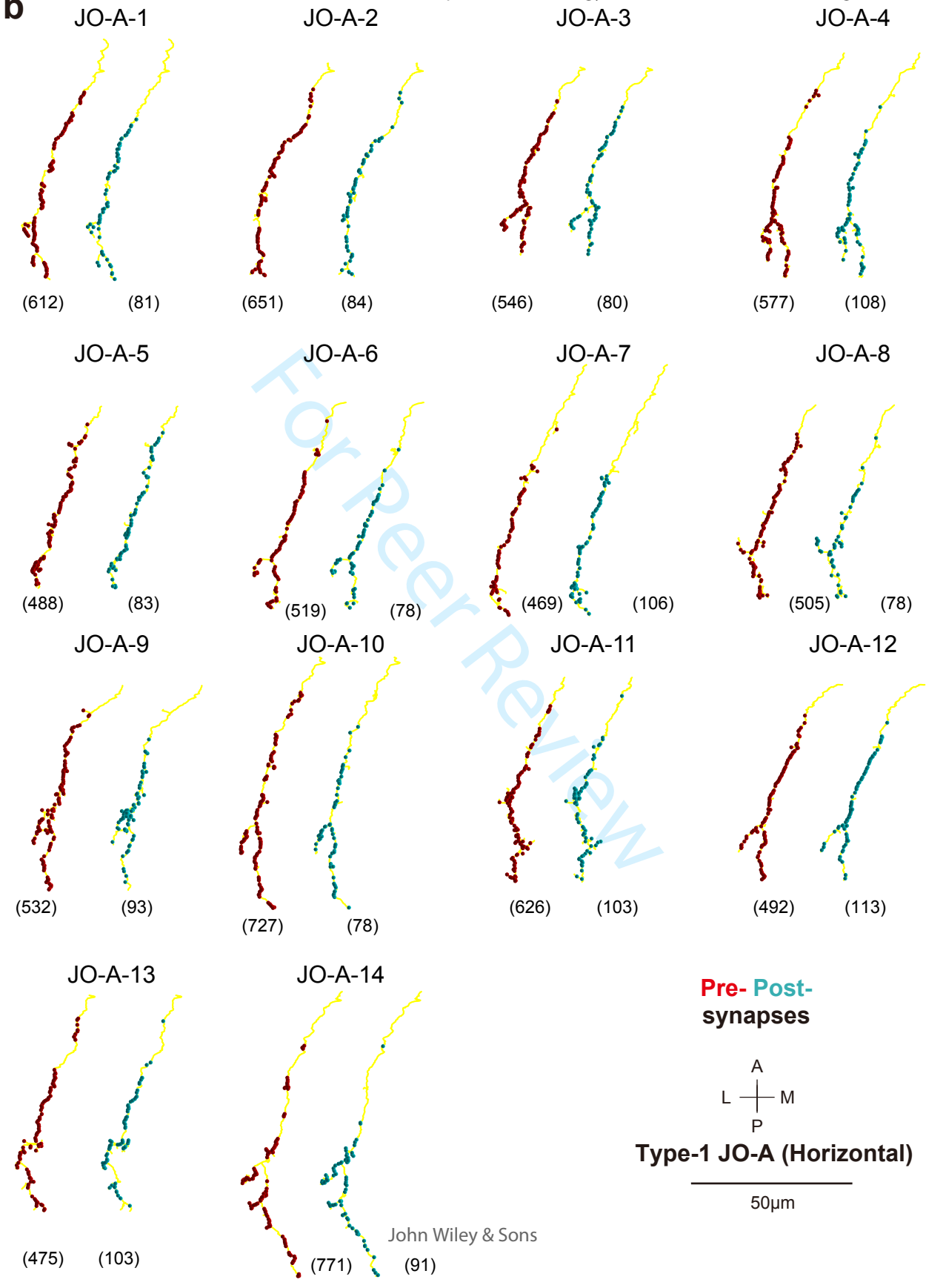
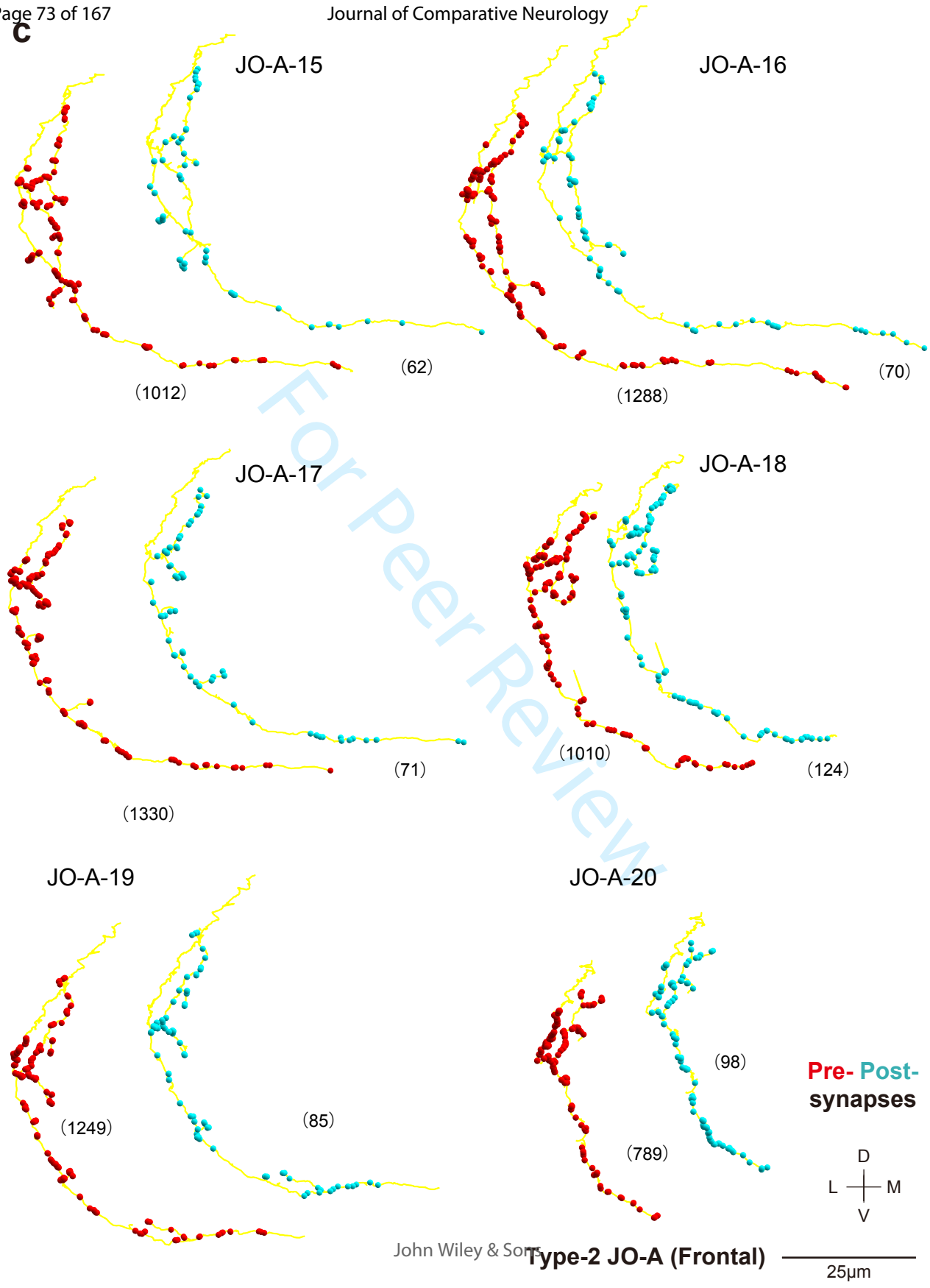
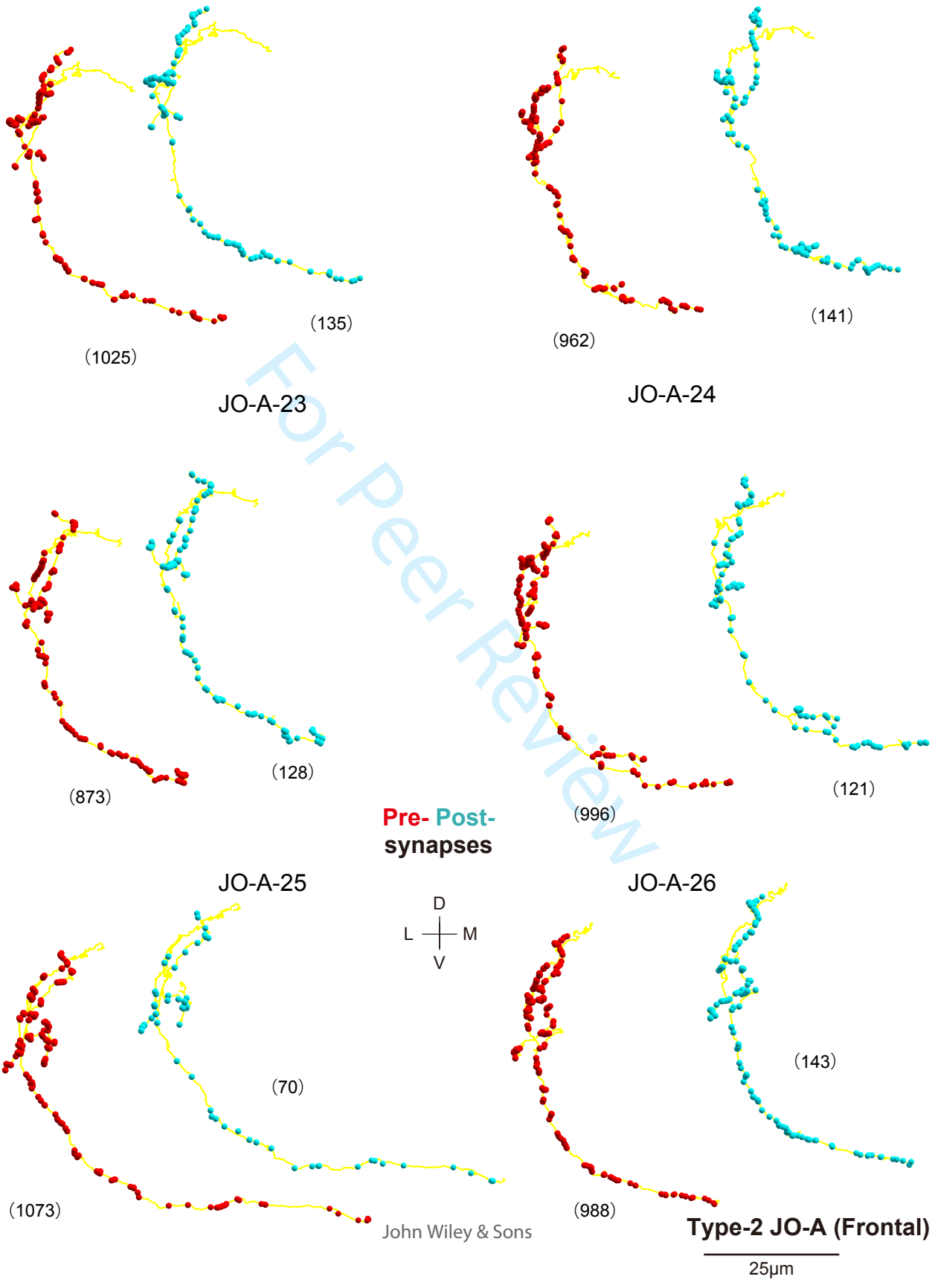
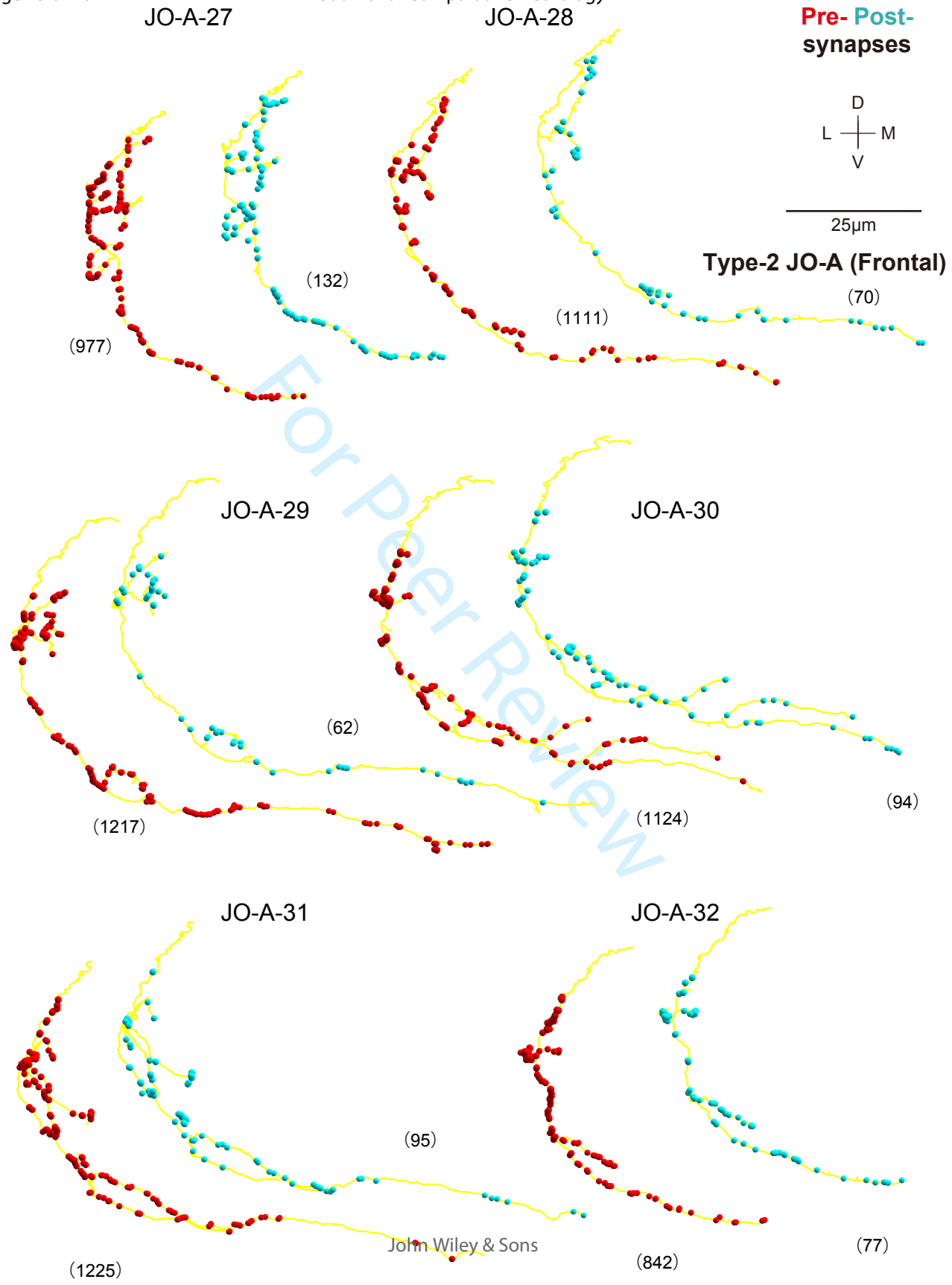


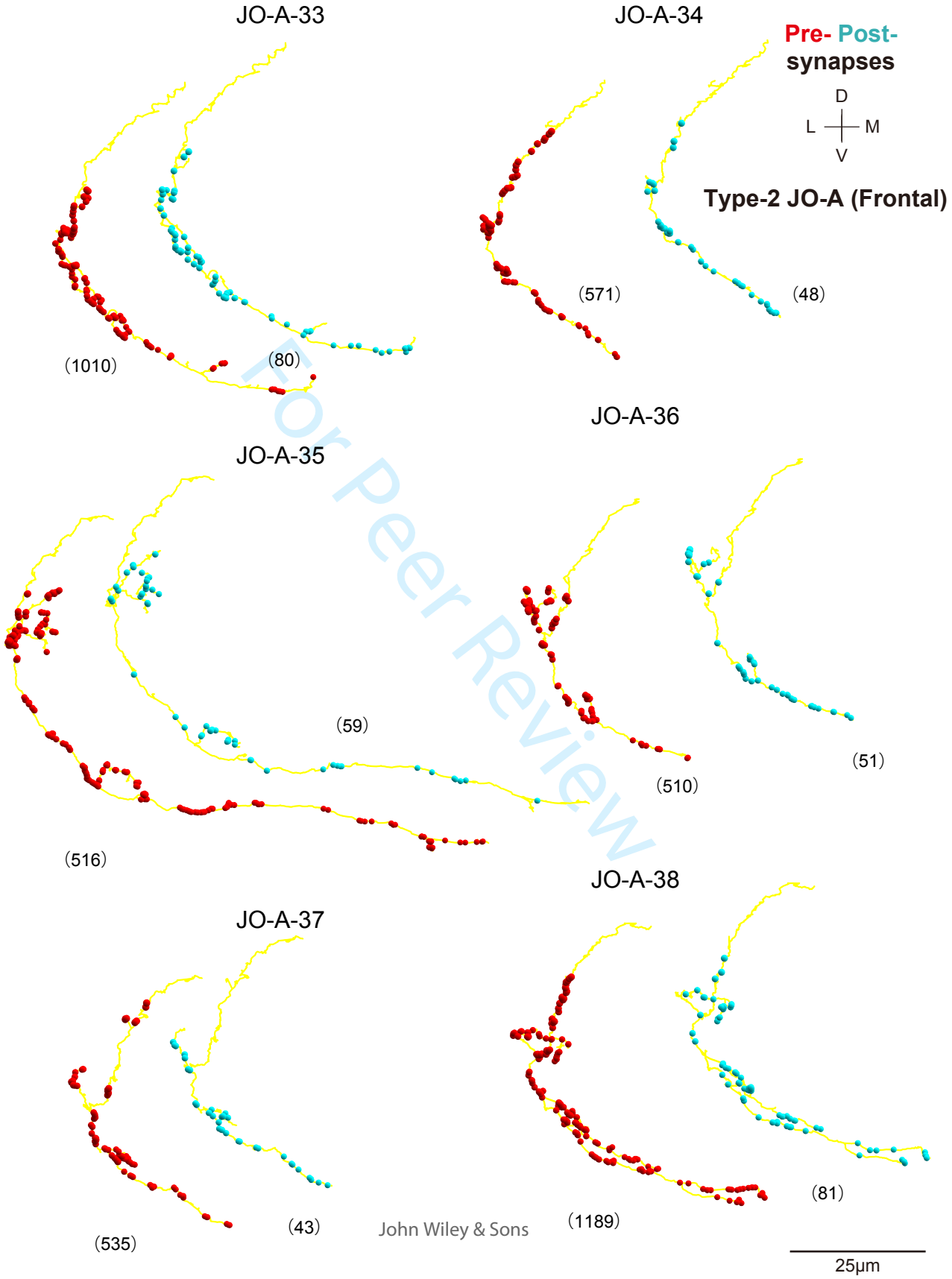
Figure S1

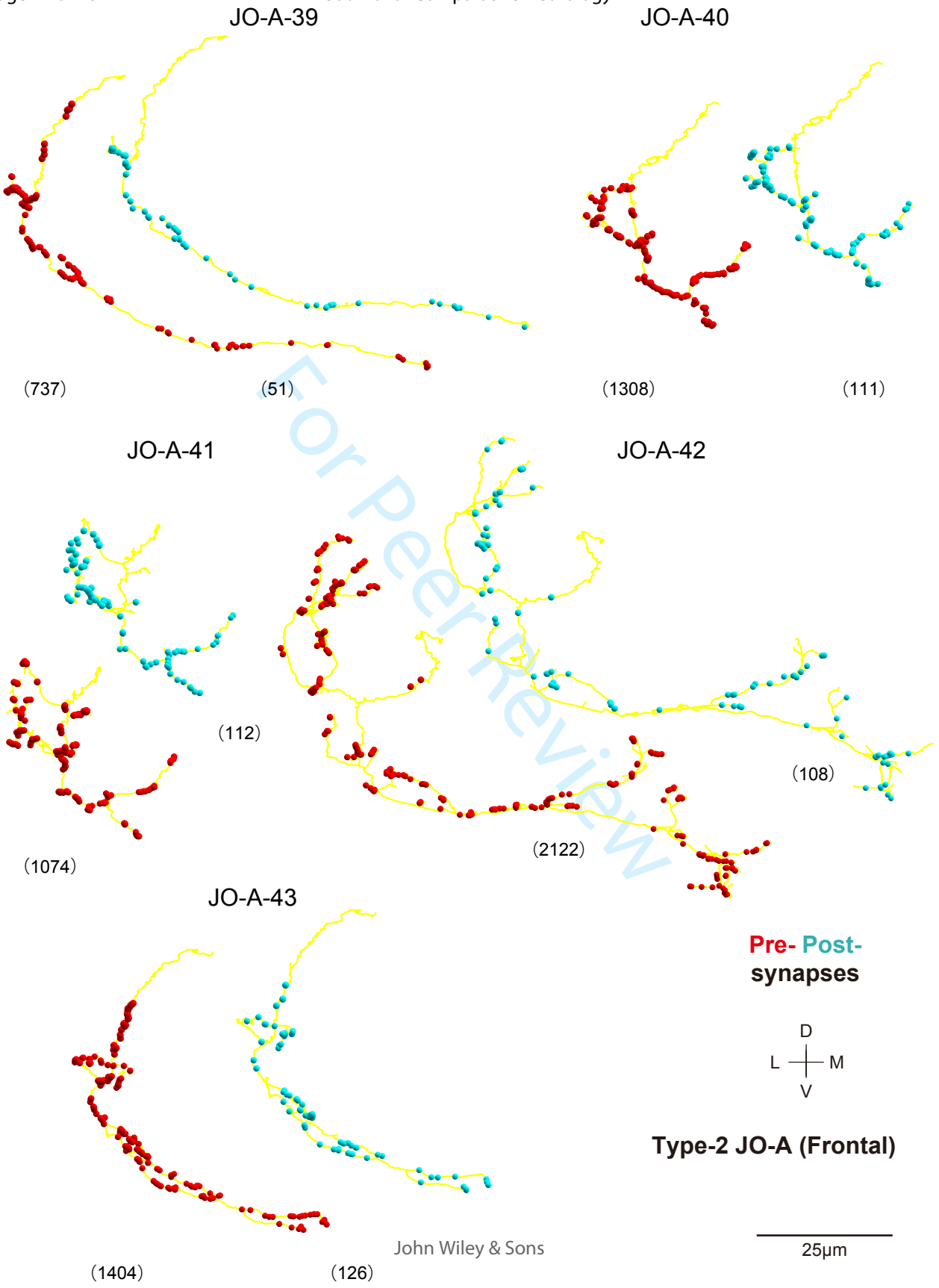


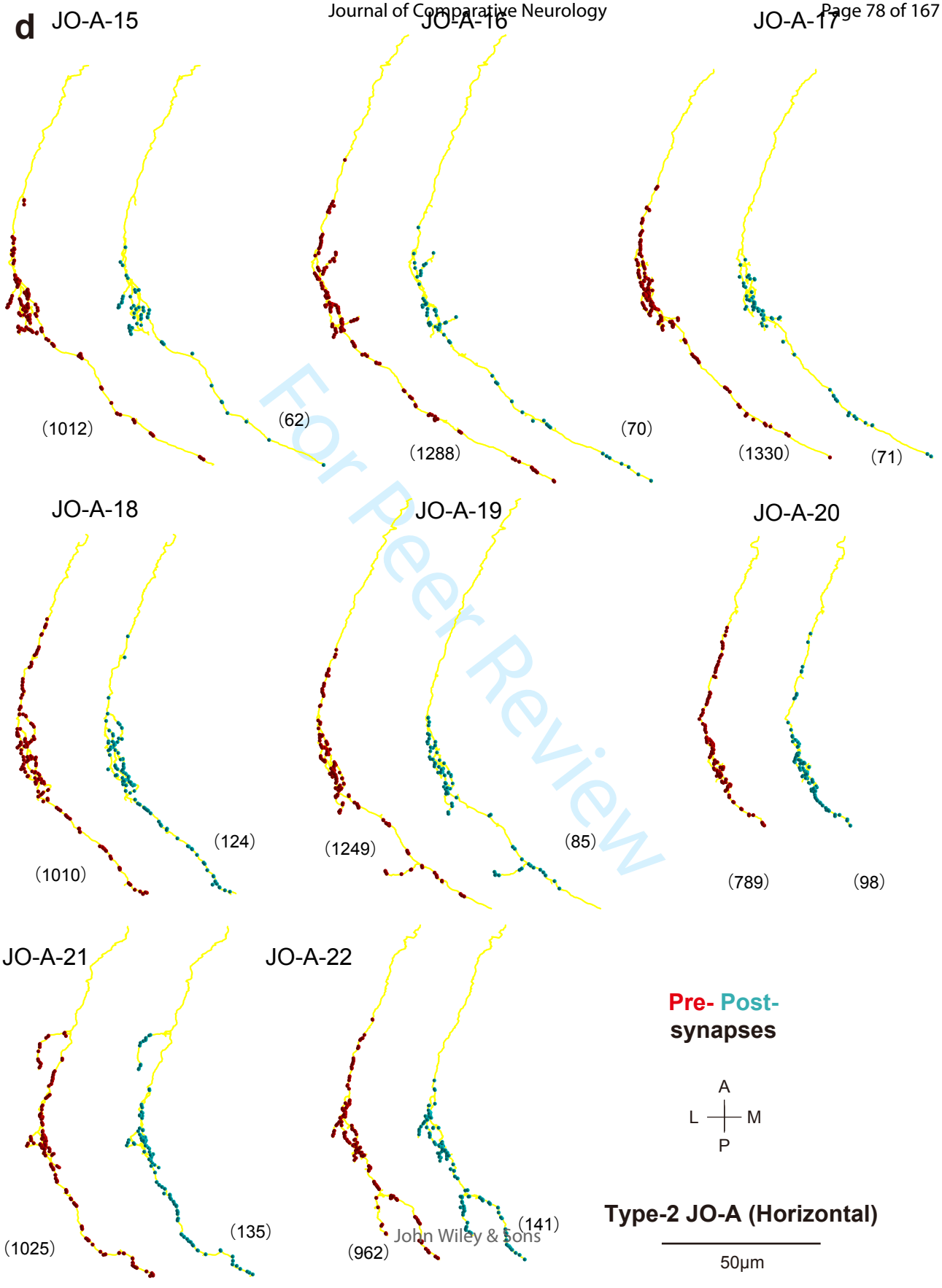


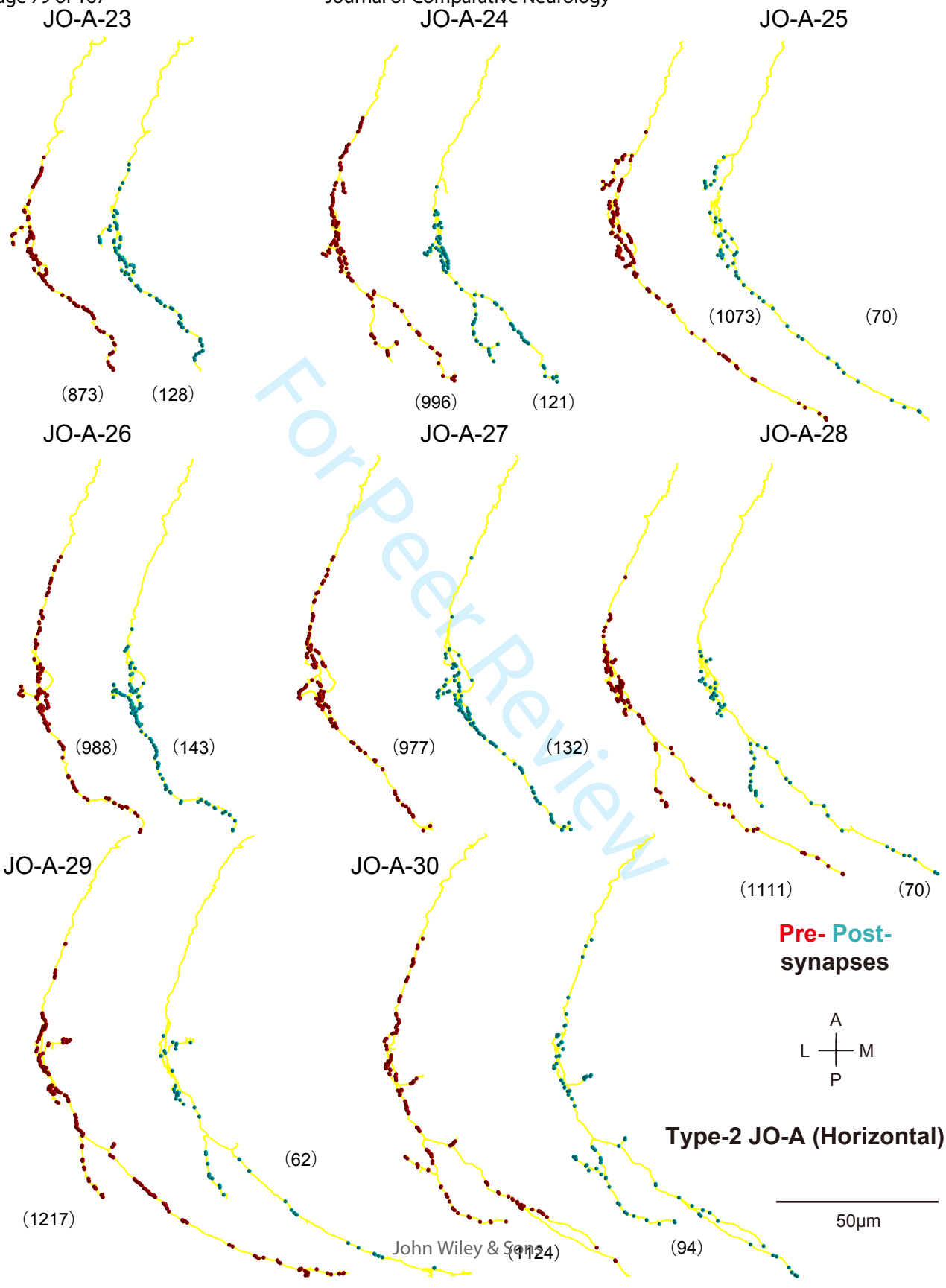


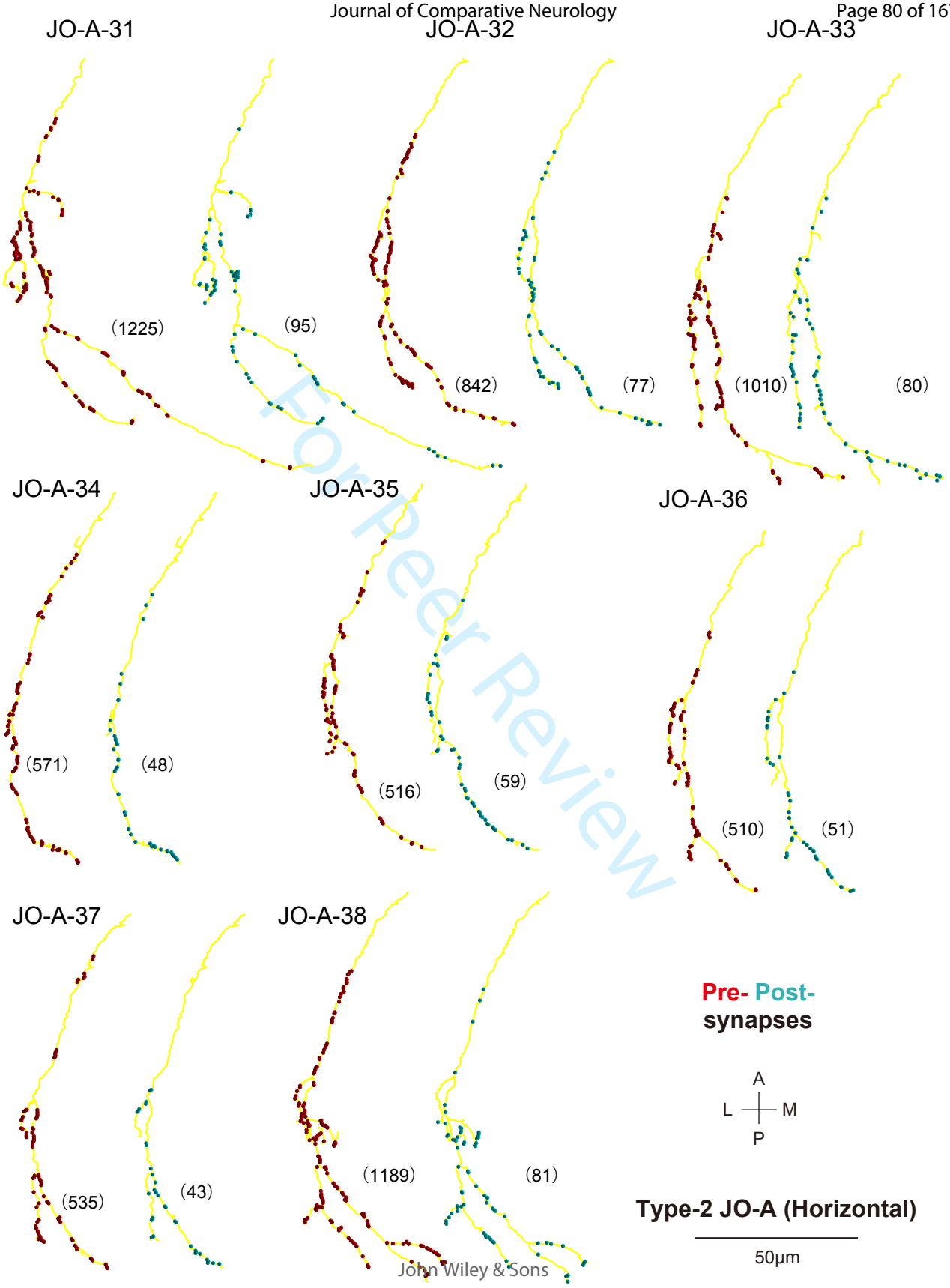


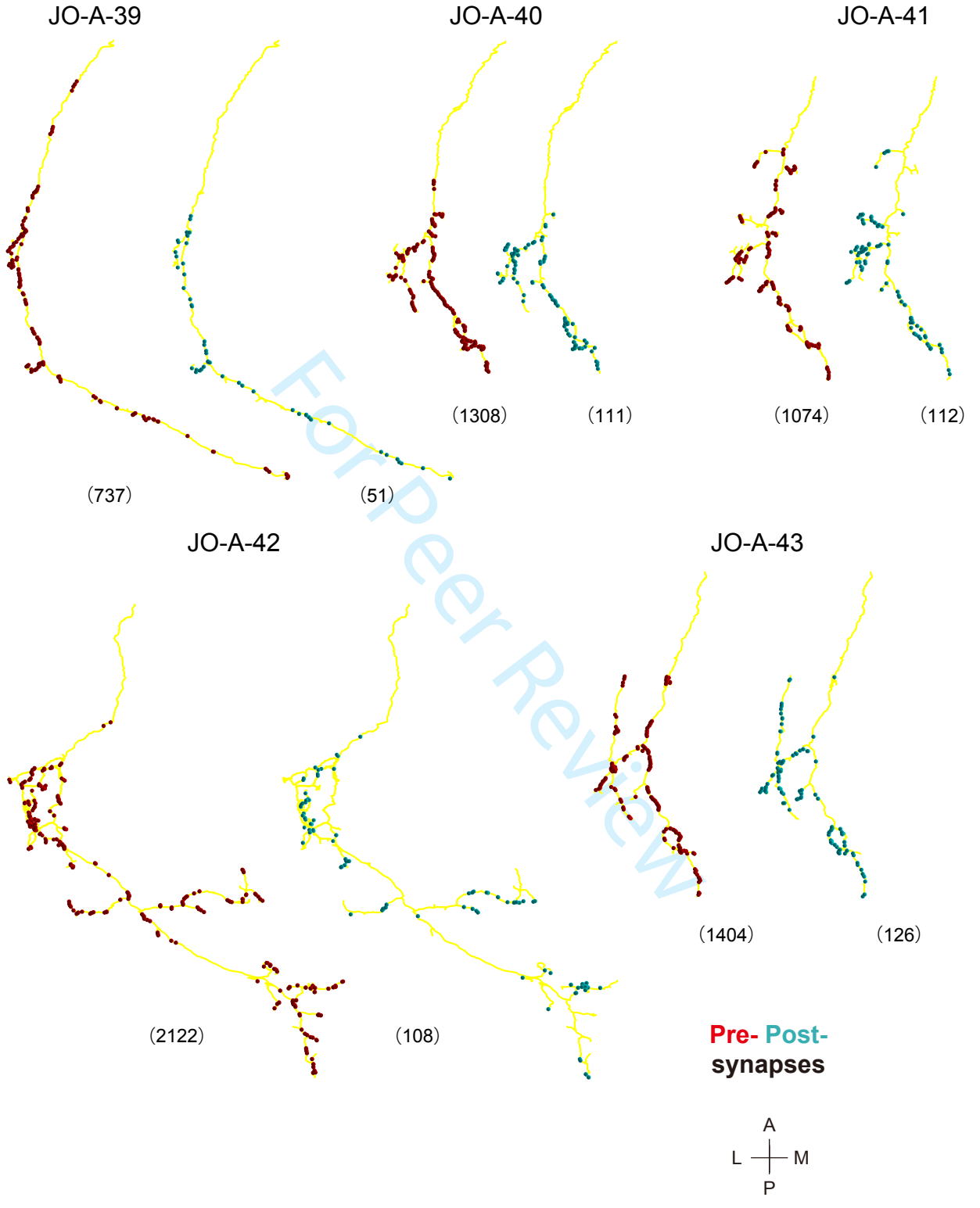












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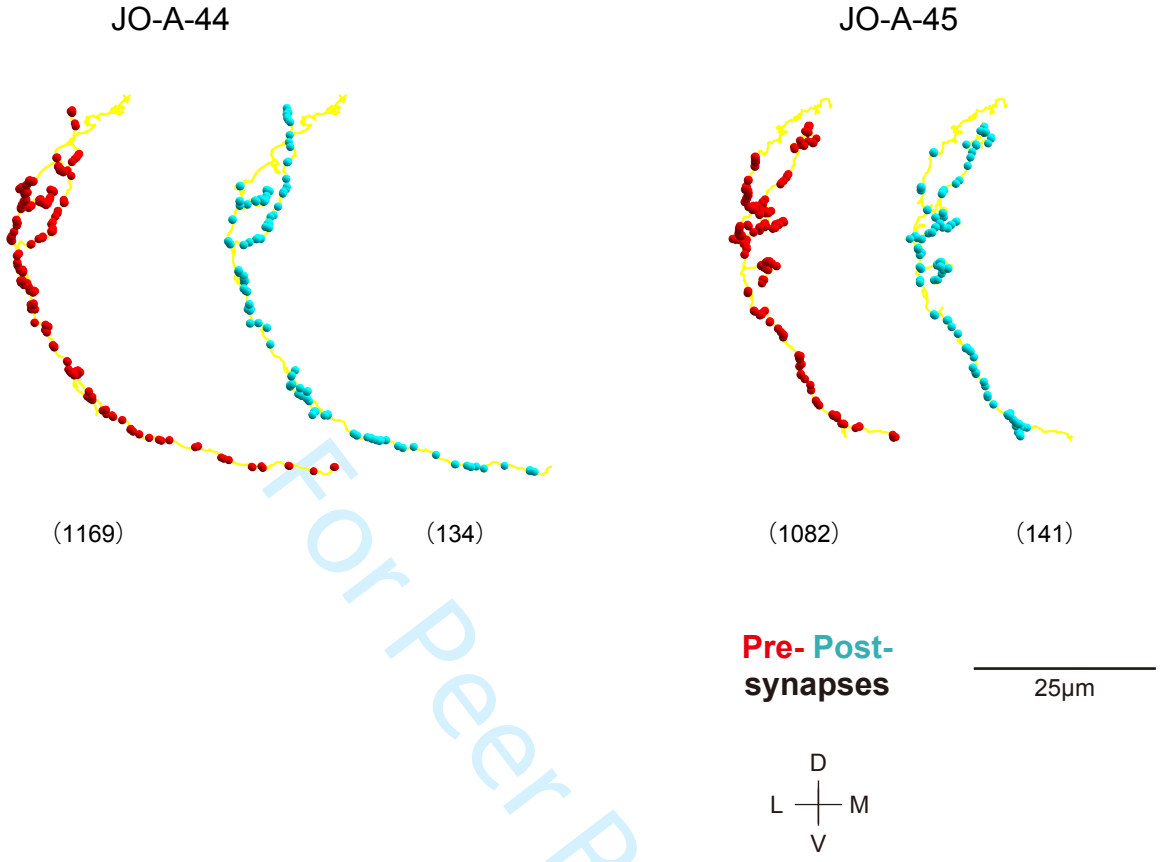
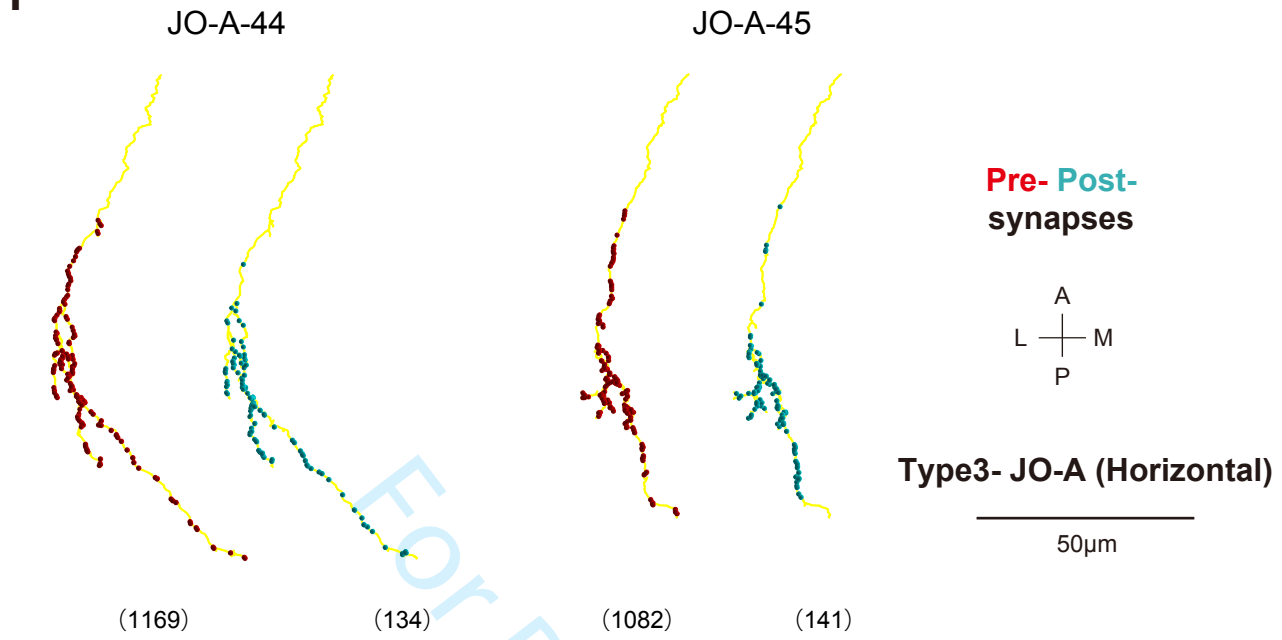




Supplementary Figure 1 | Synapse distributions along the axons of single JO-A neurons

Presynaptic (red) and postsynaptic sites (light blue) along the axon of type- 1 (a, b), 2 (c, d), and 3 (e, f) JO-A neurons (yellow). Frontal and horizontal views are shown. The upper side of the axons in the horizontal section represents the proximal side of JO neuron axons. The number in the parenthesis indicate the number of synapses in each axon. A, anterior; D, dorsal; L, lateral; M, medial; P, posterior; V, ventral.

Figure S1

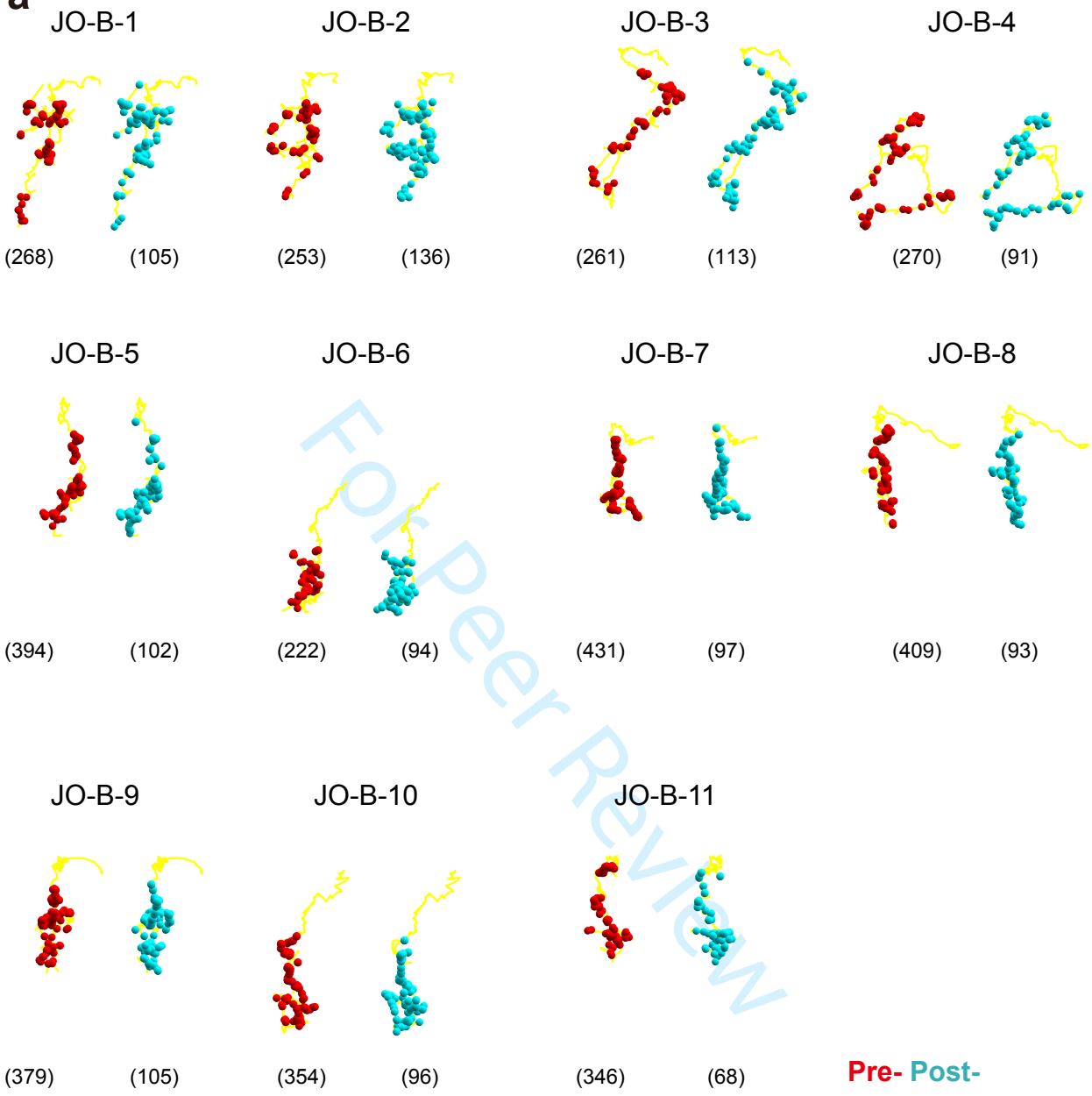
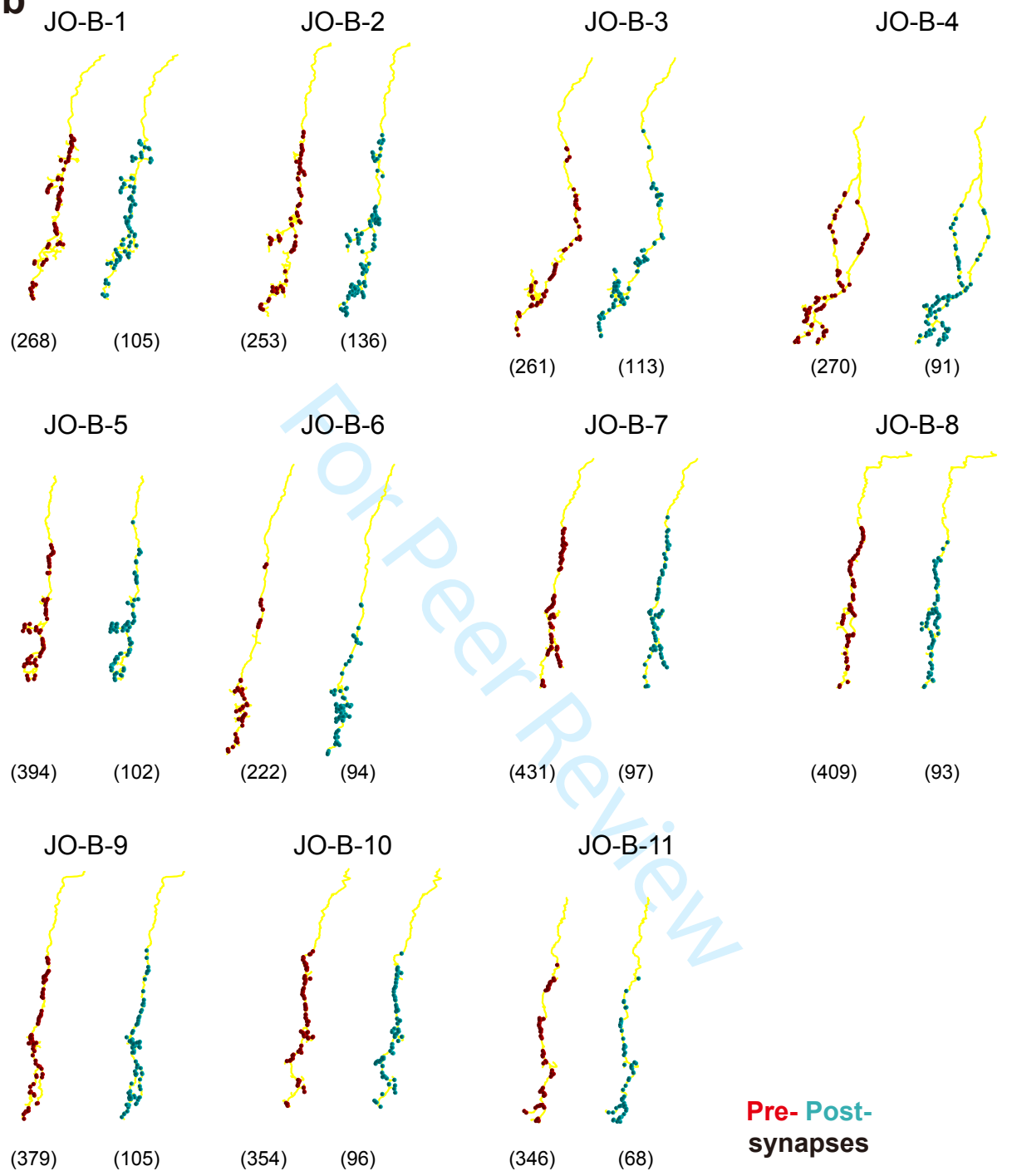
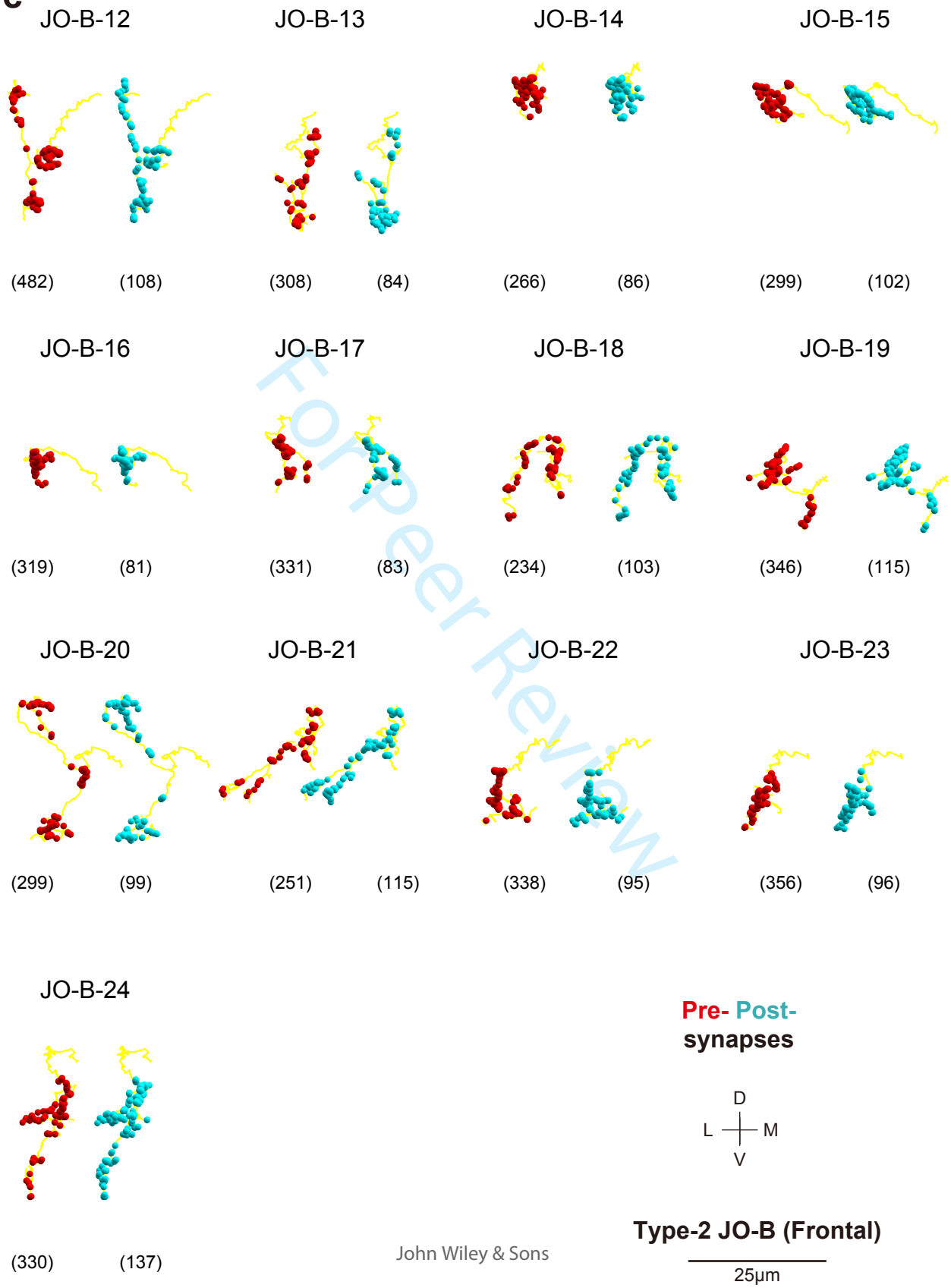


Figure S2





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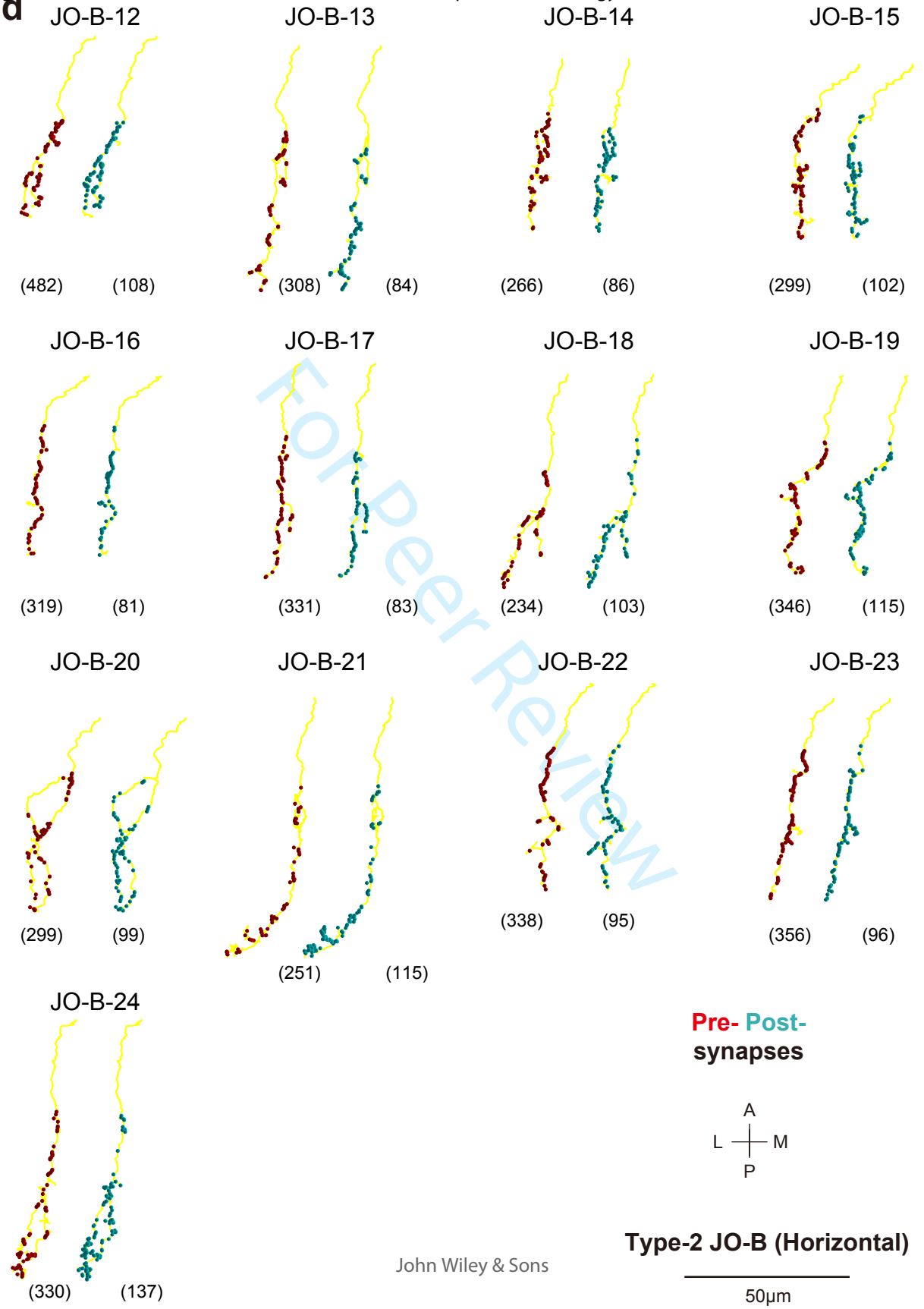

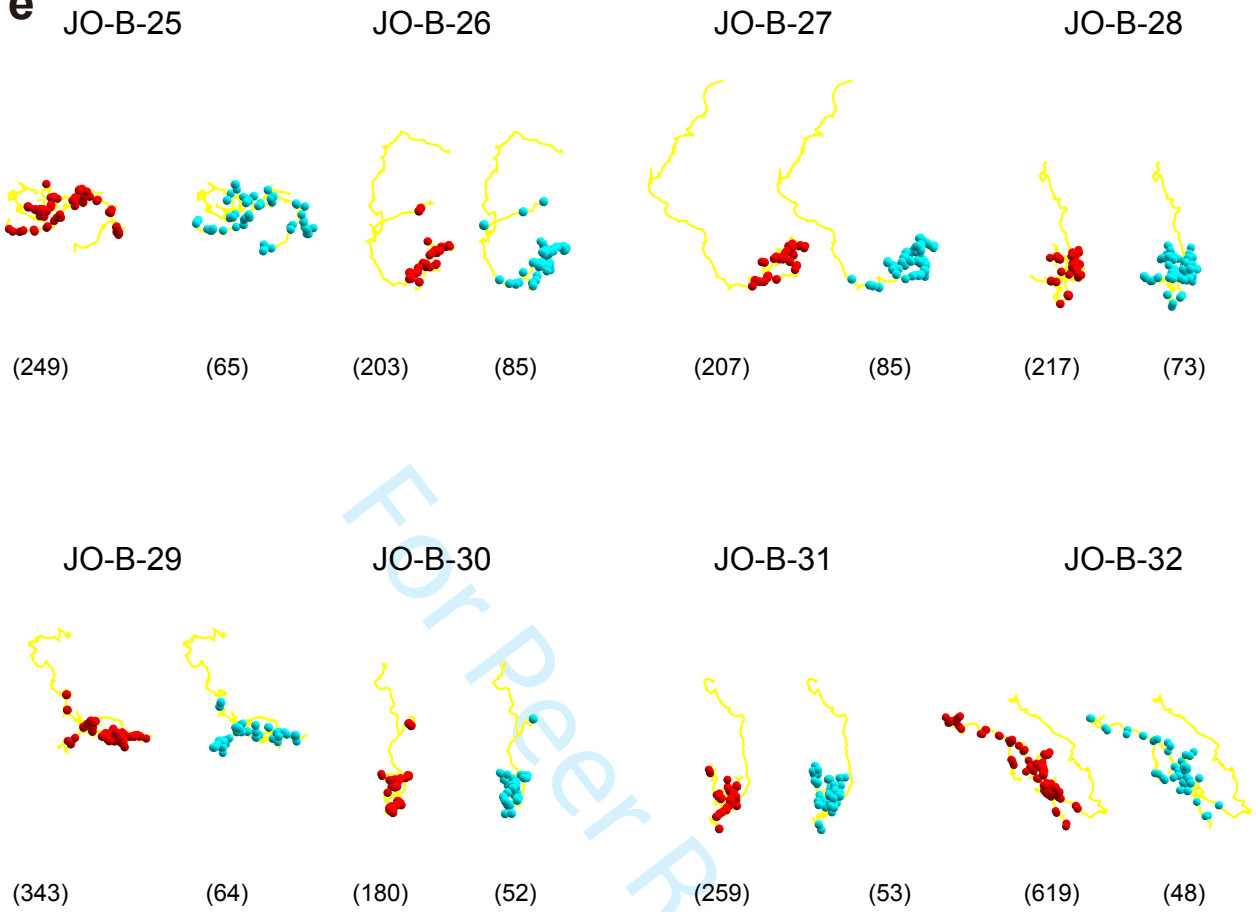

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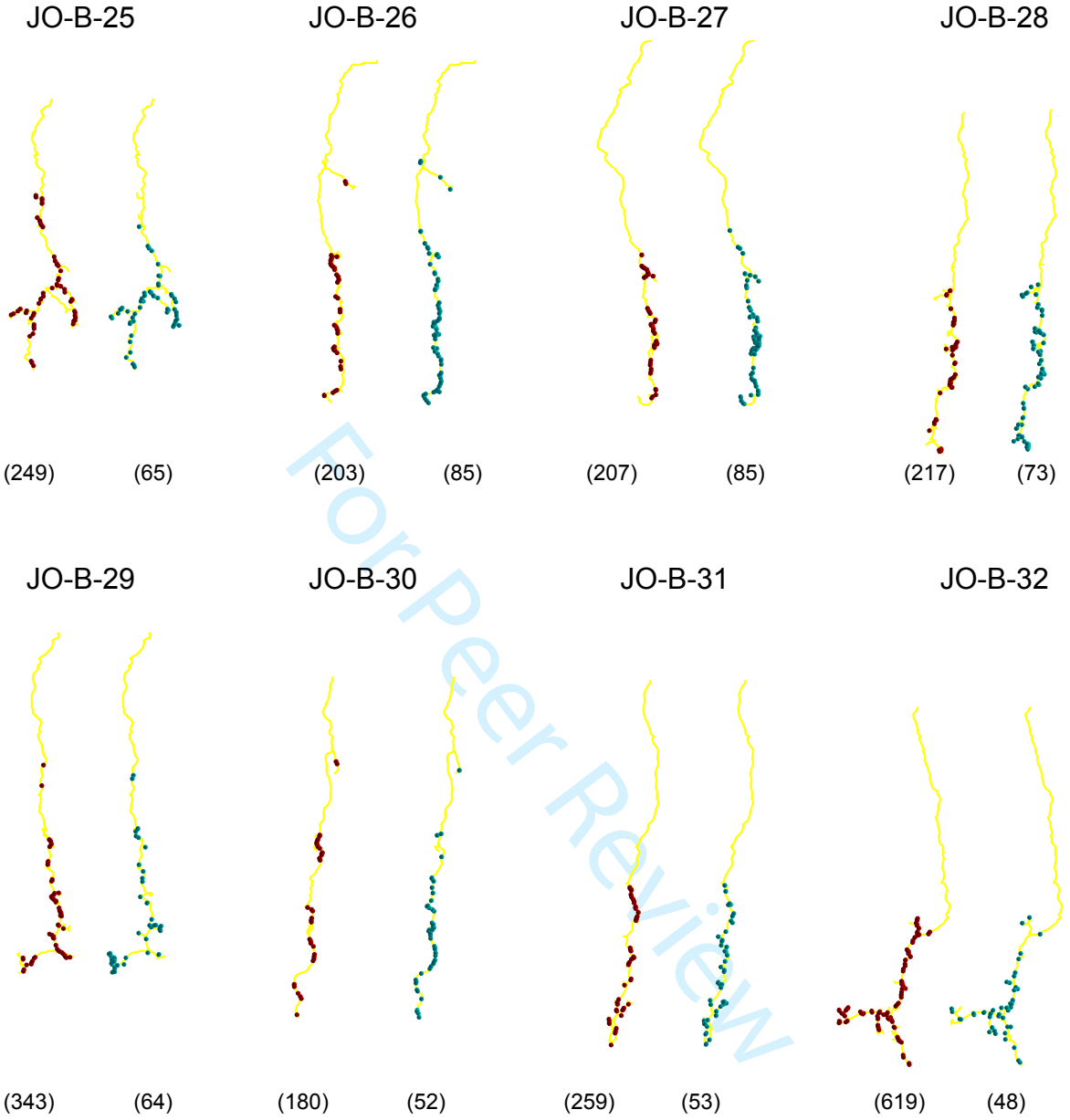

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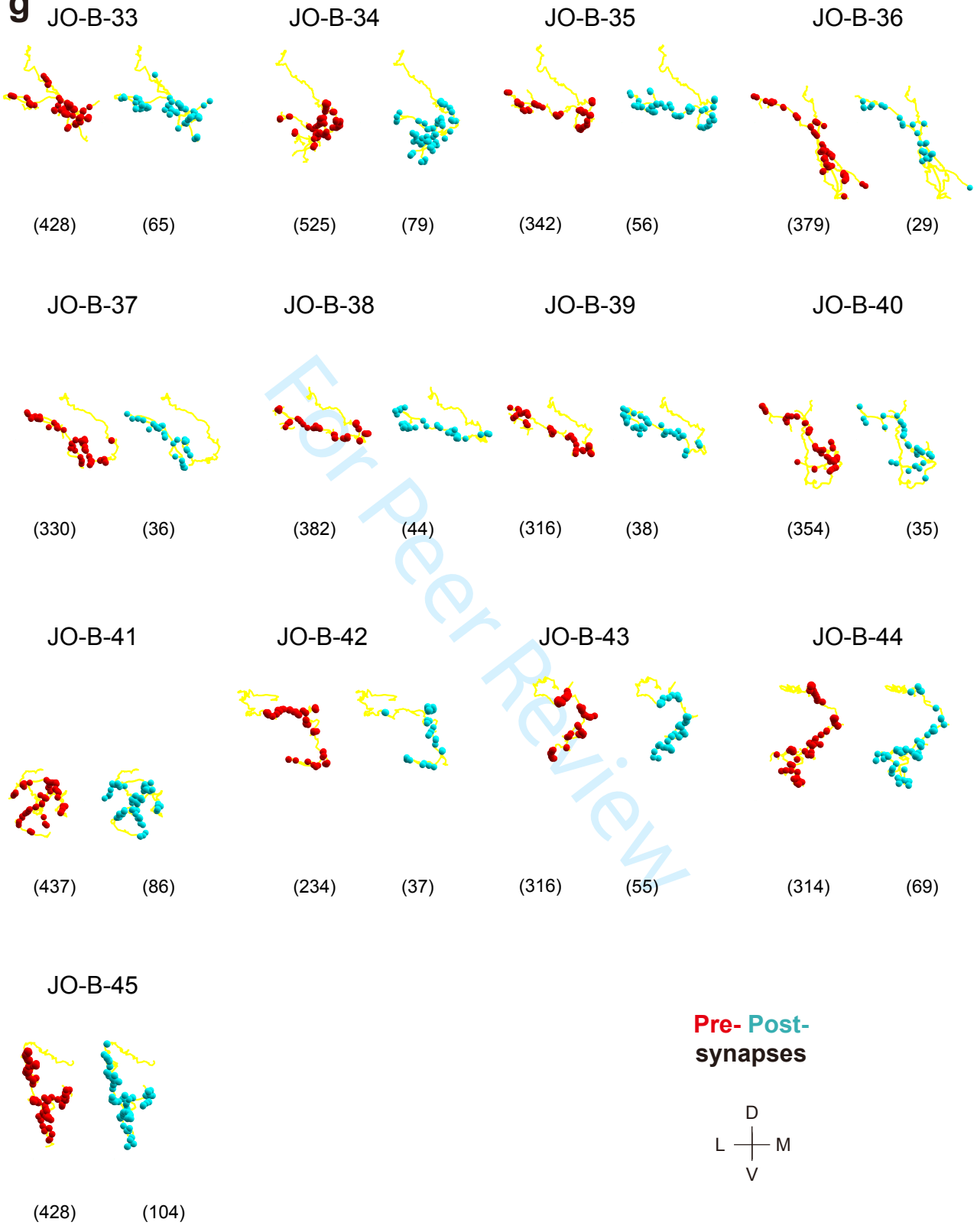

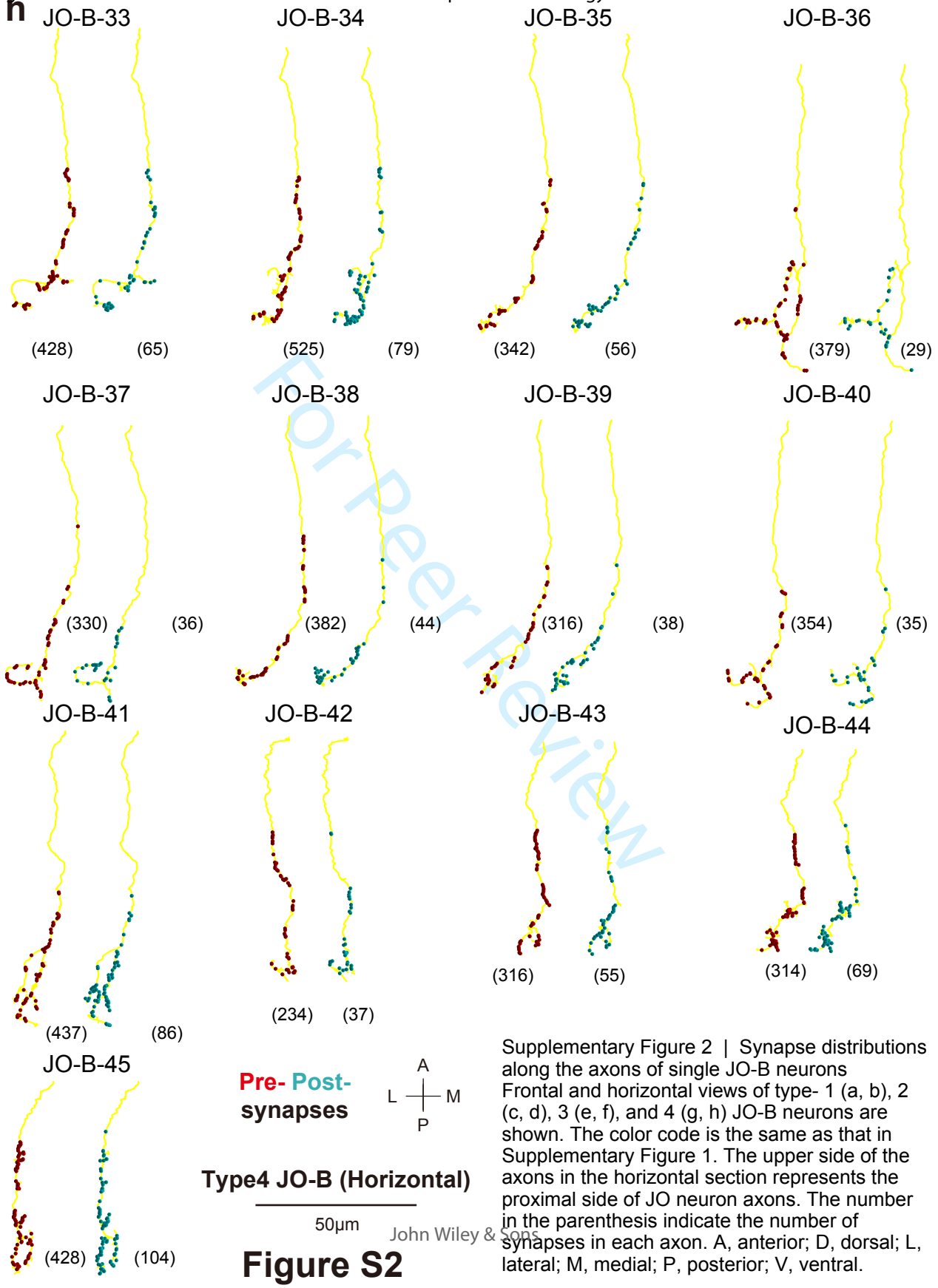

Figure S2

Supplementary Figure 2 | Synapse distributions along the axons of single JO-B neurons
Frontal and horizontal views of type- 1 (a, b), 2 (c, d), 3 (e, f), and 4 (g, h) JO-B neurons are shown. The color code is the same as that in Supplementary Figure 1. The upper side of the axons in the horizontal section represents the proximal side of JO neuron axons. The number in the parenthesis indicate the number of synapses in each axon. A, anterior; D, dorsal; L, lateral; M, medial; P, posterior; V, ventral.

Supplementary Table 1: Statistical comparisons between JO-A and JO-B neurons for the axon length

Graph type	**Neuron**		**W**	**p-value**	**Statistical method**
Normality	JO-A		0.87	1.671e-04***	Shapiro-Wilk normality test
	JO-B		0.97	0.24	
Graph type	**Neuron**			**p-value**	**Statistical method**
Homoscedasticity	JO-A *vs* JO-B			9.62e-07***	Modified robust Brown-Forsythe Levene-type test
Graph type	**Neuron**	**Df**		**p-value**	**Statistical method**
Median Comparison	JO-A *vs* JO-B	52.12		4.15e-07***	Brunner-Munzel Test

Modified robust Brown-Forsythe Levene-type test is based on the absolute deviations from the median.
Axons are significantly longer in JO-A than in JO-B.
Asterisks indicate statistical significance: ***p<0.001

Supplementary Table 2: Statistical comparisons between JO-A and JO-B neurons for the number of presynaptic sites

Graph type	**Neuron**		**p-value**	**Statistical method**
Normality	JO-A		9.88e-04***	Shapiro-Wilk normality test
	JO-B		5.34e-02	
Graph type	**Neuron**		**p-value**	**Statistical method**
Homoscedasticity	JO-A *vs* JO-B		1.96e-09***	Modified robust Brown-Forsythe Levene-type test
Graph type	**Neuron**	**Df**	**p-value**	**Statistical method**
Median Comparison	JO-A *vs* JO-B	56.09	<2.2e-16***	Shapiro Brunner-Munzel Test

Modified robust Brown-Forsythe Levene-type test is based on the absolute deviations from the median.
JO-A neuron axons have more presynaptic sites than JO-B neuron axons.
Asterisks indicate statistical significance: ***p<0.001

Supplementary Table 3: Statistical comparisons between JO-A and JO-B neurons for presynaptic sites / µm

Graph type	**Neuron**	**W**	**p-value**	**Statistical method**
Normality	JO-A	0.97	0.41	Shapiro-Wilk normality test
	JO-B	0.94	2.70e-02*	
Graph type	**Neuron**		**p-value**	**Statistical method**
Homoscedasticity	JO-A *vs* JO-B		0.33	Modified robust Brown-Forsythe Levene-type test
Graph type	**Neuron**	**W**	**p-value**	**Statistical method**
Median Comparison	JO-A *vs* JO-B	1928.5	< 2.2e-16***	Exact Wilcoxon rank sum test

Modified robust Brown-Forsythe Levene-type test is based on the absolute deviations from the median.
The number of presynaptic sites/µm is significantly larger in JO-A than in JO-B.
Asterisks indicate statistical significance: ***p<0.001, p*<0.05

Supplementary Table 4: Correlations between presynaptic and postsynaptic sites in JO-A and JO-B neurons

Graph type	Neuron				p-value	Statistical method
Normality	JO-A, pre				9.88e-04***	Shapiro-Wilk normality test
	JO-A, post				0.21	
	JO-B, pre				0.05	
	JO-B, post				0.22	
Graph type	**Neuron**	**r *or* rs**	**t**	**df**	**p-value**	**Statistical method**
Correlation between pre and post	JO-A	0.20			0.19	Spearman's rank correlation
	JO-B	-0.07	-0.49	43	0.63	Pearsons's product-moment correlation

r and rs represent the correlation coefficient in the Spearman's rank correlation test and the Pearsons's product-moment correlation test, respectively. Pre, presynapses; post, postsynapses.
Asterisks indicate statistical significance: ***p<0.001

Supplementary Table 5: Statistical comparisons between JO-A and JO-B neurons for the number of postsynaptic sites

	Graph type	Neuron		W	p-value	Statistical method
Table 1	Normality	JO-A		0.97	0.21	Shapiro-Wilk normality test
		JO-B		0.97	0.22	
	Graph type	**Neuron**	**Df**	**F**	**p-value**	**Statistical method**
	Homo scedasticity	JO-A *vs* JO-B	44 (num) 44 (denom)	1.03	0.93	F test
	Graph type	**Neuron**	**Df**	**T**	**p-value**	**Statistical method**
	Mean Comparison	JO-A *vs* JO-B	88	2.09	0.04*	Two Sample t-test

JO-A neuron axons have more postsynaptic sites than JO-B neuron axons.
Asterisks indicate statistical significance: p*<0.05

Supplementary Table 6: Statistical comparisons between JO-A and JO-B neurons for postsynaptic sites /µm

Graph type	Neuron	W	p-value	Statistical method
Normality	JO-A	0.93	1.10e-02*	Shapiro-Wilk normality test
	JO-B	0.97	0.34	
Graph type	**Neuron**		**p-value**	**Statistical method**
Homo scedasticity	JO-A *vs* JO-B		0.57	Modified robust Brown-Forsythe Levene-type test
Graph type	**Neuron**	**W**	**p-value**	**Statistical method**
Median Comparison	JO-A *vs* JO-B	669.5	5.28e-03**	Exact Wilcoxon rank sum test

Modified robust Brown-Forsythe Levene-type test is based on the absolute deviations from the median.
The number of postsynaptic sites/µm is significantly larger in JO-B than in JO-A.
Asterisks indicate statistical significance: **p<0.01, p*<0.05

Supplementary Table 7: Statistical comparisons of the depth of postsynaptic sites within different types of JO-A neurons

	Graph type	Neuron		W	p-value	Statistical method
Fig. 7	Normality	Type 1		0.99	7.17e-06***	Shapiro-Wilk normality test
		Type 2		0.99	6.03e-10***	
		Type 3		0.97	2.16e-05***	
	Graph type	**Neuron**			**p-value**	**Statistical method**
	Homo scedasticity	Within types			< 2.2e-16***	Modified robust Brown-Forsythe Levene-type test
	Graph type	**Neuron**	**Df**		**F value**	**Statistical method**
	Median Comparison	Type 1 *vs* 2	3899.2		< 2.2e-16***	Brunner-Munzel Test
		Type 1 *vs* 3	296.32		< 2.2e-16***	
		Type 2 *vs* 3	451.92		0.85	

The depths of postsynaptic sites are 81.34 ± 12.95 in type 1, 118.02 ± 15.98 in type 2, and 118.10 ± 10.33 µm in type 3 (mean ± standard deviation).
Modified robust Brown-Forsythe Levene-type test is based on the absolute deviations from the median.
Significance level was set at 0.017 after applying Bonferroni correction.
The location of postsynaptic sites is significantly deeper in type-1 and 2 JO-A than in type 3.
Asterisks indicate statistical significance: ***p<0.001

Supplementary Table 8: Statistical comparisons of the depth of presynaptic sites within different types of JO-A neurons

	Graph type	Neuron		D	p-value	Statistical method
Fig. 7	Normality	Type 1		5.15e-02	< 2.2e-16***	One-sample Kolmogorov-Smirnov test
		Type 2		6.42e-02	< 2.2e-16***	
		Type 3		7.97e-02	7.55e-13***	
	Graph type	**Neuron**			**p-value**	**Statistical method**
	Homo scedasticity	Within types			< 2.2e-16***	Modified robust Brown-Forsythe Levene-type test
	Graph type	**Neuron**	**Df**		**F value**	**Statistical method**
	Median Comparison	Type 1 *vs* 2	22909		< 2.2e-16***	Brunner-Munzel Test
		Type 1 *vs* 3	2782.3		< 2.2e-16***	
		Type 2 *vs* 3	2918.1		0.29	

The depths of presynaptic sites are 81.91 ± 14.86 in type 1, 113.30 ± 18.04 in type 2, and 113.22 ± 13.51 µm in type 3 (mean ± standard deviation).
Modified robust Brown-Forsythe Levene-type test is based on the absolute deviations from the median.
Significance level was set at 0.017 after applying Bonferroni correction.
The location of presynaptic sites is significantly deeper in type-1 and 2 JO-A than in type 3.
Asterisks indicate statistical significance: ***p<0.001

Supplementary Table 9: Statistical comparisons between type-1 and 2 JO-A neurons for the axon length

Graph type	Neuron	W	p-value	Statistical method
Normality	Type 1	0.90	0.11	Shapiro-Wilk normality test
	Type 2	0.74	9.49e-06***	
Graph type	**Neuron**		**p-value**	**Statistical method**
Homoscedasticity	Type 1 *vs* 2		6.65e-02	Modified robust Brown-Forsythe Levene-type test
Graph type	**Neuron**	**W**	**p-value**	**Statistical method**
Median Comparison	Type 1 *vs* 2	0	2.55e-11***	Exact Wilcoxon rank sum test

Modified robust Brown-Forsythe Levene-type test is based on the absolute deviations from the median.
Axon length is significantly longer in type-2 JO-A than in type 1. Because there were only two type-3 JO-A neurons, we did not include them in the statistical tests.
Asterisks indicate statistical significance: ***p<0.001

Supplementary Table 10: Statistical comparisons between type-1 and 2 JO-A neurons for the depth of axonal projections

Graph type	Neuron		W	p-value	Statistical method
Normality	Type 1		0.93	0.29	Shapiro-Wilk normality test
	Type 2		0.95	0.24	
Graph type	**Neuron**	**Df**	**F**	**p-value**	**Statistical method**
Homoscedasticity	Type 1 *vs* 2	13 (num) 28 (denom)	0.45	0.13	F test
Graph type	**Neuron**	**Df**	**T**	**p-value**	**Statistical method**
Mean Comparison	Type 1 *vs* 2	41	-15.36	<2.2e-16***	Two Sample t-test

Projection depth is significantly deeper in type-2 JO-A than type 1. Because there were only two type-3 JO-A neurons, we did not include them in the statistical tests.
Asterisks indicate statistical significance: ***p<0.001

Supplementary Table 11: Correlations between the number of pre and post - synaptic sites and axon length within JO-A neurons

Graph type	Neuron		p-value	Statistical method
Normality	Presynapse of JO-A		9.88e-04***	Shapiro-Wilk normality test
	Postsynapse of JO-A		0.21	
	Axon length of JO-A		1.67e-04***	
Graph type	**Neuron**	**rs**	**p-value**	**Statistical method**
Correlation	Presynapses and length	0.86	2.04e-14***	Spearman's rank correlation
	Postsynapses and length	0.02	0.90	

Asterisks indicate statistical significance: ***p<0.001

Supplementary Table 12: Statistical comparisons of the axon length within different types of JO-B neurons

Graph type	Neuron		W		p-value	Statistical method
Normality	Type 1		0.89		0.13	Shapiro-Wilk normality test
	Type 2		0.94		0.51	
	Type 3		0.89		0.23	
	Type 4		0.97		0.85	
Graph type	**Neuron**	**Df**			**p-value**	**Statistical method**
Homoscedasticity	Within types	3			0.79	Bartlett test
Graph type	**Neuron**	**Df**	**Sq**	**F value**	**Pr (>F)**	**Statistical method**
Comparison of means	Within types	3	2564 (Sum) 854.6 (Mean)	0.56	0.69	One-way ANOVA
	Residuals	41	50802 (Sum) 1239.1 (Mean)			

No significant difference within types of JO-B neurons.

Supplementary Table 13: Statistical comparisons of the depth of axonal projections within different types of JO-B neurons

Graph type	Neuron		W	p-value	Statistical method
Normality	Type 1		0.87	6.72e-02	Shapiro-Wilk normality test
	Type 2		0.95	0.59	
	Type 3		0.94	0.57	
	Type 4		0.78	3.80e-03**	
Graph type	**Neuron**			**p-value**	**Statistical method**
Homo scedasticity	Within types			4.99e-2	Modified robust Brown-Forsythe Levene-type test
Graph type	**Neuron**	**Df**		**F value**	**Statistical method**
Median Comparison	Type 1 *vs* 2	21.77		0.76	Brunner-Munzel Test
	Type 1 *vs* 3	12.89		2.41e-10***	
	Type 1 *vs* 4	15.19		5.41e-03**	
	Type 2 *vs* 3	17.68		< 2.2e-16***	
	Type 2 *vs* 4	14.39		3.61e-03**	
	Type 3 *vs* 4	18.63		0.72	

Type-1 and 2 JO-B neurons terminate more anteriorly than type- 3 and 4.
Modified robust Brown-Forsythe Levene-type test is based on the absolute deviations from the median.
Significance level was set at 0.0083 after applying Bonferroni correction.
Asterisks indicate statistical significance: ***p<0.001, **p<0.01

Supplementary Table 14: Statistical comparisons of the number of postsynapses within different types of JO-B neurons

Graph type	**Neuron**			**W**	**p-value**	**Statistical method**
Normality	Type 1			0.92	0.30	Shapiro-Wilk normality test
	Type 2			0.93	0.31	
	Type 3			0.90	0.32	
	Type 4			0.92	0.25	
Graph type	**Neuron**	**Df**			**p-value**	**Statistical method**
Homoscedasticity	Within types	3			0.44	Bartlett test
Graph type	**Neuron**	**Df**	**Sq**	**F value**	**Pr (>F)**	**Statistical method**
Comparison of means	Within Type	3	18579 (Sum) 6193 (Mean)	18.65	8.77e-08***	One-way ANOVA
	Residuals	41	13618 (Sum) 332 (Mean)			
Graph type	**Neuron**	**diff**	**lwr**	**Upr**	**P adj**	**Statistical method**
Multiple comparison	Type 2 *vs* 1	0.31	-19.68	20.30	1.00	Tukey multiple comparison
	Type 3 *vs* 1	-34.38	-57.05	11.70	1.19e-03**	
	Type 4 *vs* 1	-43.62	-63.61	-23.62	4.20e-06***	
	Type 3 *vs* 2	-34.68	-56.61	-12.75	7.03e-04***	
	Type 4 *vs* 2	-43.92	-63.06	-24.78	1.60e-06***	
	Type 4 *vs* 3	-9.24	-31.17	12.69	0.67	

Type-1 and 2 JO-B neuron axons have more postsynaptic sites than type- 3 and 4.
Asterisks indicate statistical significance: ***p<0.001, **p<0.01

Supplementary Table 15: Statistical comparisons of the depth of postsynaptic sites within different types of JO-B neurons

	Graph type	Neuron		W	p-value	Statistical method
Fig. 10	Normality	Type 1		0.99	1.07e-08***	Shapiro-Wilk normality test
		Type 2		0.98	1.50e-10***	
		Type 3		0.99	5.25e-05***	
		Type 4		0.90	< 2.2e-16***	
	Graph type	**Neuron**			**p-value**	**Statistical method**
	Homoscedasticity	Within types			< 2.2e-16***	Modified robust Brown-Forsythe Levene-type test
	Graph type	**Neuron**	**Df**		**F value**	**Statistical method**
	Median Comparison	Type 1 *vs* 2	2335.6		0.31	Brunner-Munzel Test
		Type 1 *vs* 3	914.17		< 2.2e-16***	
		Type 1 *vs* 4	1016.4		< 2.2e-16***	
		Type 2 *vs* 3	929.66		< 2.2e-16***	
		Type 2 *vs* 4	996.94		< 2.2e-16***	
		Type 3 *vs* 4	1163.3		1.29e-03**	

The depths of postsynaptic sites are 92.41 ± 15.19 in type 1, 92.09 ± 15.24 in type 2, 115.77 ± 16.49 in type 3, and 118.41 ± 23.02 µm in type 4 (mean ± standard deviation).
The location of postsynaptic sites is significantly deeper in type- 3 and 4 JO-A than in type 1 and 2, and in type-4 JO-A than in type 3.
Modified robust Brown-Forsythe Levene-type test is based on the absolute deviations from the median.
Significance level set at 0.0083 after applying Bonferroni correction
Asterisks indicate statistical significance: ***p<0.001, **p<0.01

Supplementary Table 16: Statistical comparisons of the depth of presynaptic sites within different types of JO-B neurons

	Graph type	Neuron		W	p-value	Statistical method
	Normality	Type 1		0.97	< 2.2e-16***	Shapiro-Wilk normality test
		Type 2		0.97	< 2.2e-16***	
		Type 3		0.97	< 2.2e-16***	
		Type 4		0.92	< 2.2e-16***	
	Graph type	**Neuron**			**p-value**	**Statistical method**
	Homoscedasticity	Within types			< 2.2e-16***	Modified robust Brown-Forsythe Levene-type test
	Graph type	**Neuron**	**Df**		**F value**	**Statistical method**
Fig. 10	Median Comparison	Type 1 *vs* 2	7398.5		9.84e-02	Brunner-Munzel Test
		Type 1 *vs* 3	4293.3		< 2.2e-16***	
		Type 1 *vs* 4	7438.1		< 2.2e-16***	
		Type 2 *vs* 3	4335.4		< 2.2e-16***	
		Type 2 *vs* 4	7266.4		< 2.2e-16***	
		Type 3 *vs* 4	5921.7		6.97e-05***	

The depths of presynaptic sites are 86.89 ± 16.03 in type 1, 86.25 ± 15.64 in type 2, 117.93 ± 18.51 in type 3, and 118.41 ± 23.47 µm in type 4 (mean ± standard deviation).

The location of presynaptic sites is significantly deeper in type- 3 and 4 JO-A than in type 1 and 2, and in type-4 JO-A than in type 3.

Modified robust Brown-Forsythe Levene-type test is based on the absolute deviations from the median.

Significance level was set at 0.0083 after applying Bonferroni correction.

Asterisks indicate statistical significance: ***p<0.001

Supplementary Table 17: Statistical comparisons of the numbers of AMMC-B1 neurons labelled as monosynaptic downstream of JO-A and JO-B neurons

	Graph type	Neuron		W	p-value	Statistical method
Fig. 14	Normality	JO-A		0.86	8.83e-02	Shapiro-Wilk normality test
		JO-B		0.92	0.43	
	Graph type	**Neuron**	**Df**	**F**	**p-value**	**Statistical method**
	Homoscedasticity	JO-A *vs* JO-B	8 (num) 7 (denom)	0.92	0.90	F test
	Graph type	**Neuron**	**Df**	**T**	**p-value**	**Statistical method**
	Mean Comparison	JO-A *vs* JO-B	15	-7.56	1.72e-06***	Two Sample t-test

trans-Tango of JO-B neurons have more labeled AMMC-B1 neurons than that of JO-A neurons.

Asterisks indicate statistical significance: ***p<0.001