



















Journal of Comparative Neurology



Type-2 JO-A (Horizontal)





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



Type-1 JO-B (Frontal)

25µm

Figure S2



Type-1 JO-B (Horizontal)

•		Jou	rnal of Compai	Page 86 of 167			
JO-E	3-12	JO-B-1	3	JO-B-	-14	JO-E	3-15
œ	et et			×.	şş.	*	
(482)	(108)	(308)	(84)	(266)	(86)	(299)	(102)
JO-I	3-16	JO-B-1	7	JO-B-	-18	JO-E	3-19
ţ	ر ب ر کر		À	Į.	JA REAL	Se,	- Story
(319)	(81)	(331)	(83)	(234)	(103)	(346)	(115)
JO-E	3-20	JO-B-21		JO-B-	22	JO-E	3-23
		a a a for the second	Ŕ	Je j		× i	
(299)	(99)	(251)	(115)	(338)	(95)	(356)	(96)
JO-E	3-24				Pre- syna	Post- pses □ ↓ M V	
(330)	(137)		John Wile	y & Sons	Type-2 J	Ο-Β (Fron 25μm	tal)





Pre- Postsynapses

D L — M V

John Wiley & Sons

25µm

Type-3 JO-B (Frontal)



Pre- Postsynapses

A L – M P

Type-3 JO-B (Horizontal)





D L — M V

(428) (104)

Type-4 JO-B (Frontal)

25µm



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

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

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***	Shanira Wills normality tost	
Normanty	JO-B		5.34e-02	Shapiro-wirk normanty test	
Graph type	Neuron	(p-value	Statistical method	
Homosoadastiaity	JO-A vs	-	1 062 00***	Modified robust Brown-	
Homoscedasticity	JO-B		1.908-09***	Forsythe Levene-type test	
Graph	Nouron	Df	n valuo	Statistical mathed	
type	rearon	DI	p-value	Statistical method	
Median	JO-A vs	56.00	<2.2.2.16***	Shapira Druppar Munzal Test	
Comparison	JO-B	30.09	~2.2 e -10***	Shapho Brunner-Munzer 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

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

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

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

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

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

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
	Normality	JO-A		0.97	0.21	Shapiro-Wilk
	normanty	JO-B		0.97	0.22	normality test
Table 1	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	Т	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*	Shanira Wills normality tast
Normanty	JO-B	0.97	0.34	Shapho-wirk hormanty test
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/ μ m is significantly larger in JO-B than in JO-A. Asterisks indicate statistical significance: **p<0.01, p*<0.05

	Graph type	Neuron		W	p-value	Statistical method
		Type 1		0.99	7.17e-06***	01 . 11.11
	Normality	Type 2		0.99	6.03e-10***	Shapiro-Wilk
		Type 3		0.97	2.16e-05***	normanty test
	Graph type Neuron				p-value	Statistical method
Fig. 7	Homo scedasticity	Within types			< 2.2e-16***	Modified robust Brown-Forsythe Levene-type test
	Graph type	Neuron	Df		F value	Statistical method
		Type 1 vs 2	3899.2		< 2.2e-16***	
	Comparison	Type 1 vs 3	296.32		< 2.2e-16***	Brunner-Munzel
	Comparison	Type 2 vs 3	451.92		0.85	rest

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

The depths of postsynaptic sites are 81.34 ± 12.95 in type 1, 118.02 ± 15.98 in type 2, and $118.10 \pm$ 10.33 μ m in type 3 (mean \pm 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
		Type 1		5.15e-02	< 2.2e-16***	One-sample
	Normality	Type 2		6.42e-02	< 2.2e-16***	Kolmogorov-
		Type 3		7.97e-02	7.55e-13***	Smirnov test
	Graph type	Neuron			p-value	Statistical method
Fig. 7	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 <i>vs</i> 2	22909		< 2.2e-16***	
		Type 1 <i>vs</i> 3	2782.3		<2.2e-16***	Brunner-Munzel Test
		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 ± 14.86 13.51 μ m in type 3 (mean \pm 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

Graph type	Neuron	W	p-value	Statistical method
Normality	Type 1	0.90	0.11	Shapiro-Wilk normality
Normanty	Type 2	0.74	9.49e-06***	test
Graph type	Neuron		p-value	Statistical method
Homoscodasticity	Type 1		6 652 02	Modified robust Brown-
Homoscedasticity	<i>vs</i> 2		0.036-02	Forsythe Levene-type test
Graph type	Neuron	W	p-value	Statistical method
Median	Type 1	0	255 11***	Exact Wilcoxon
Comparison	vs 2	0	2.336-11	rank sum test

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

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
NT 114	Type 1		0.93	0.29	Shapiro- Wilk
Normality	Type 2	9	0.95	0.24	normality test
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	Т	p-value	Statistical method
Mean Comparison	Type 1 vs 2	41	-15.36	<2.2e- 16***	Two Sample

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

Asterisks indicate statistical significance: ***p<0.001

Graph type	Neuron		W		p-value	method
	Type 1		0.89		0.13	Shapiro-
Normality	Type 2		0.94		0.51	Wilk
Normanty	Type 3		0.89		0.23	normality
	Type 4		0.97		0.85	test
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	Within types	3	2564 (Sum) 854.6 (Mean)	0.56	0.69	One-way
of means	Residuals	41	50802 (Sum) 1239.1			ANOVĂ

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

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
	Type 1		0.87	6.72e-02	
Normality	Type 2		0.95	0.59	Shapiro-Wilk
Normanty	Type 3		0.94	0.57	normality test
	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
	Type 1 vs 2	21.77		0.76	
	Type 1 vs 3	12.89		2.41e-10***	
Median	Type 1 <i>vs</i> 4	15.19		5.41e-03**	Brunner-Munzel
Comparison	Type 2 <i>vs</i> 3	17.68		< 2.2e-16***	Test
	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

Graph type	Neuron			vv	p-value	method	
	Type 1			0.92	0.30	Shapiro-	
Normality	Type 2			0.93	0.31	Wilk	
Normanty	Type 3			0.90	0.32	normality	
	Type 4			0.92	0.25	test	
Graph type	Neuron	Df			p-value	Statistical method	
Homoscedas ticity	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	
	Residuals	41	13618 (Sum) 332 (Mean)			ANOVÁ	
Graph type	Neuron	diff	lwr	Upr	P adj	Statistical method	
	Type 2 <i>vs</i> 1	0.31	-19.68	20.30	1.00		
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***	Tukey	
	Type 3 vs 2	-34.68	-56.61	-12.75	7.03e-04***	comparison	
	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		

Supplementary Table 14:Statistical comparisons of the number of postsynapseswithin different types of JO-B neuronsGraph typeNeuronWn yalueStatistical

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

r	21		1		1	
	Graph type	Neuron		W	p-value	Statistical method
-		Type 1		0.99	1.07e-08***	
	NT 114	Type 2		0.98	1.50e-10***	Shapiro-Wilk
	Normanty	Type 3		0.99	5.25e-05***	normality test
		Type 4		0.90	< 2.2e-16***	
	Graph type	Neuron			p-value	Statistical method
	Homoscedast	Within			< 2.2e-16***	Modified robust Brown-Forsythe
	ıcıty	types				Levene-type test
	Graph type	Neuron	Df		F value	Statistical method
Fig. 10	Median Comparison	Type 1 <i>vs</i> 2	2335.6		0.31	
		Type 1 <i>vs</i> 3	914.17		< 2.2e-16***	
		Type 1 <i>vs</i> 4	1016.4		< 2.2e-16***	Brunner-Munzel
		Type 2 vs 3	929.66		< 2.2e-16***	Test
		Type 2 <i>vs</i> 4	996.94		< 2.2e-16***	
		Type 3 vs 4	1163.3		1.29e-03**	

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

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 $118.41 \pm 23.02 \mu m$ in type 4 (mean \pm 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

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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
		Type 1		0.97	< 2.2e-16***	
		Type 2		0.97	< 2.2e-16***	Shapiro-Wilk
	Normanty	Type 3		0.97	< 2.2e-16***	normality test
		Type 4		0.92	< 2.2e-16***	
	Graph type	Neuron			p-value	Statistical method
	Homoscedastic	Within				Modified robust
	ity	types			<2.2e-16***	Brown-Forsythe
	ity types					Levene-type test
	Graph type	Neuron	Df		F value	Statistical method
Fig. 10	Median	Туре	7398.5		9.84e-02	
-		1 vs 2				
		Туре	4293.3		< 2 20-16***	
		1 vs 3			< 2.26-10	
		Туре	7438.1	90	< 2 2e-16***	Brunner-Munzel
		1 vs 4			< 2.20-10	
0	Comparison	Туре	4335.4		< 2.2e-16***	Test
		2 vs 3				
		Туре	7266.4			
		2 vs 4			~ 2.2e-10	
		Туре	5021 7		(07-05***	
		3 vs 4	5921.7		0.970-03	

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 $118.41 \pm 23.47 \mu m$ in type 4 (mean \pm 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
	Normality	JO-A		0.86	8.83e-02	Shapiro-Wilk
	Normanty	JO-B		0.92	0.43	normality test
Fig. 14	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	Т	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

_____nore la .icance: ****p.