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Supplemental Material

Developmental Neurotoxicity of the Harmful Algal Bloom Toxin Domoic Acid: Cellular and Molecular Mechanisms Underlying Altered Behavior in the Zebrafish Model

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Additional File- Excel and Code Document

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SUPPLEMENTARY MATERIAL

Supplemental Material: Reagents and Sources

Reagent	**Source**
Domoic acid	Sigma-Aldrich, St. Louis, MO (Catalog # D6152)
RNA isolation kit	Zymo Direct-Zol kit (Catalog # R2062)
TruSeq total RNA library kit	Illumina

Supplemental Material: Equipment, Sources, and Settings

Equipment	**Sources and Settings**
glass capillary tubes (058 mm inner diameter)	World Precision Instruments (1B100F-4)
pipette puller	Sutter Instrument, model p-30 (heat 750, pull= 0)
microinjector	Narishige IM-300
speaker	Visaton BG20-8 8" Full-Range Speaker with Whizzer Cone (#292-548)
amplifier	WONDOM 100W TDA7498 Class-D Amplifier Board (#320-303)
pulse generator	PulsePal, Sansworks
high-speed video camera	Edgertronic
accelerometer	PCB Piezotronics (model W356B11)
pulse conditioner	PCB Piezotronics (model 480B31)
analog filter	Krohn-Hite Corporation (model 3382)
data acquisition board	National Instruments (model USB-6251)
confocal microscope	Zeiss (LSM-710 and LSM-780)
40x water objective	Zeiss C- Apochromat, NA= 1.1
inverted epifluorescence microscope	Zeiss
Bioanalyzer	Agilent technologies, CA

Table S1: Study-specific metrics

General assay	Description	Reference	DomA doses	Injection times	Assessment times	Total number of fish
Behavior	Startle responsiveness	Fig. 2	0, 0.09, 0.13, 0.14, 0.18 ng	1, 2, 4 dpf	7 dpf	1,865
Behavior	Startle kinematics	Fig. 3	0, 0.09, 0.13, 0.14, 0.18 ng	1, 2, 4 dpf	7 dpf	983
Behavior	Startle kinematics	Fig. 4	0, 0.09, 0.13, 0.14, 0.18 ng	1, 2, 4 dpf	7 dpf	1552
Microscopy	Myelin sheath imaging (confocal)	Fig. 5	0, 0.13- 0.14 ng	1, 1.5, 2, 2.5, 4 dpf	5 dpf	433
Microscopy	Myelin sheath imaging (widefield epifluorescence)	Fig. 6	0, 0.09, 0.13, 0.14, 0.18 ng	1, 1.5, 2, 2.5, 3, 4 dpf	5 dpf	2841
Microscopy	Myelin sheath imaging (widefield epifluorescence)	Fig. 7	0, 0.09, 0.13, 0.14, 0.18 ng	1, 2, 4 dpf	6, 7 dpf	876
Microscopy	Time-lapse myelin sheath imaging (confocal)	Fig. 8	0, 0.14 ng	2 dpf	2.5-3 dpf	11
RNASeq	-	Fig. 9	0, 0.14 ng	2 dpf	3, 5, 7 dpf	3 pools of 6 per treatment
Morphological assessment	Swim bladder analysis	Fig. S3	0, 0.09, 0.13, 0.14, 0.18 ng	1, 2, 4 dpf	5-7 dpf	450
Behavior	Acute neurotoxicity	Fig. S3	0, 0.09, 0.14, 0.18 ng	1, 2, 4 dpf	daily post-injection to 5 dpf	3020
Mortality	-	Table S4	0, 0.09, 0.13, 0.14, 0.18 ng	1, 1.5, 2, 2.5, 3, 4 dpf	5 dpf	3937
Morphological assessment	Opaque brain analysis	Table S3	0, 0.09, 0.13, 0.14, 0.18 ng	1, 1.5, 2, 2.5, 3, 4 dpf	5 dpf	3886

Notes: Total number of fish indicate single animals unless otherwise noted. DomA= domoic acid, dpf= days post fertilization.

Table S2: Swim bladder analysis: percent of fish with inflated swim bladders by day injected and dose

		Control	DomA (nominal dose)			
		0 ng	**0.09 ng**	**0.13 ng**	**0.14 ng**	**0.18 ng**
Day injected	**1 dpf**	95% (19/20)			100% (24/24)	
	2 dpf	97.9% ± 1.42 (24/25, 8/8, 26/26, 22/24, 47/47, 4/4)		11.11% (1/9)	18.0% ± 2.64 (6/38, 2/12, 3/26, 5/27, 11/40)	10% (1/10)
	4 dpf	100%, 100% (25/25, 10/10)	83.63% ± 5.85 (17/19, 7/9)		84% (21/25)	90%, 50% (9/10, 6/12)

Notes: Percent of fish with inflated swim bladders were recorded after imaging fish from 5-7 days post fertilization (dpf). Percent shown is the mean percent of the population with inflated swim bladders ± standard error of the mean if there were more than two repeated trials. Otherwise, values from a single trial are listed. Within the parentheses, the ratio of the number of fish that have inflated swim bladders to the total number of fish are listed, with individual trials separated by commas. Fish injected at 4 dpf with 0.18 ng domoic acid (DomA) that also had 'opaque brains' were excluded from this analysis. See Table S3 for opaque brain phenotype breakdown. See Figure S3B for the graphical representation of this table. DomA= domoic acid, dpf= days post fertilization, SE= standard error of the mean.

Table S3: Opaque brains in *Tg(mbp:EGFP-CAAX)* larvae exposed to domoic acid by intravenous injection

Day injected	Control	DomA (nominal dose injected)			
	0 ng	**0.09 ng**	**0.13 ng**	**0.14 ng**	**0.18 ng**
1 dpf	0% ± 0 (0/90, 0/71, 0/62, 0/64, 0/69, 0/62)	0% ± 0 (0/56, 0/67, 0/67)	0% (0/63)		0% (0/73, 0/63, 0/68, 0/75, 0/31)
1.5 dpf	0% (0/40)				0% 0/46
2 dpf	0% ± 0 (0/39, 0/70, 0/70, 0/64, 0/73, 0/58, 0/48, 0/96, 0/20, 0/45, 0/38, 0/58, 0/58)	0% ± 0 (0/64, 0/83, 0/76)	0%, 0% (0/66, 0/48)	0% ± 0 (0/47, 0/20, 0/27, 0/46, 0/36, 0/47)	0% ± 0 (0/74, 0/53, 0/60, 0/57, 0/72, 0/55)
2.5 dpf	0% 0/20			0% 0/21	
3 dpf	0% (0/6)			0% 0/14	
4 dpf	0% ± 0 (0/41, 0/65, 0/77, 0/71, 0/27, 0/32, 0/69)	0.5% ± 0.5 (0/76, 0/67, 1/72)	0%, 2.2% (0/91, 1/46)	(0%, 5%) (2/40, 0/41)	40.6% ± 9.5 (20/43, 26/80, 51/80, 14/72)

Notes: Percent of fish with opaque brains was recorded at 5 days post fertilization (dpf). Percent shown is the mean percent of fish with opaque brains ± the standard error of the mean when there were two or more repeated trials. Otherwise, values from a single trial are listed. Within the parentheses, the ratios of the number of fish that had opaque brains to the total number of fish are listed, with individual trials separated by commas.
DomA= domoic acid, dpf= days post fertilization, SE= standard error of the mean.

Table S4: Mortality in *Tg(mbp:EGFP-CAAX)* larvae exposed to domoic acid by intravenous injection

Day injected	Control	DomA (nominal dose)			
	0 ng	**0.09 ng**	**0.13 ng**	**0.14 ng**	**0.18 ng**
1 dpf	0.4% ± 0.4 (2/92, 0/71, 0/62, 0/64, 0/69,0/62)	1.9% ± 1.3 (0/56, 1/68, 3/70)	3.1% (2/65)		0.8% ± 0.5 (2/75, 0/63,1/69,0/75,0/31)
1.5 dpf	0% (0/40)				0% (0/46)
2 dpf	1.2% ± 0.7 (4/43, 2/72, 0/70, 0/64, 0/73, 0/58, 0/48, 0/96, 0/20, 0/45, 0/38, 0/58, 2/60)	0.4% ± 0.4 (0/64, 0/83, 1/77)	2.9%, 0% (2/68, 0/48)	0% ± 0 (0/47, 0/20, 0/27, 0/46, 0/36, 0/47)	6.2% ± 3.9 (6/80, 17/70, 0/60, 0/57, 0/72, 3/58)
2.5 dpf	0% (0/20)			0% (0/21)	
3 dpf	0% (0/6)			0% (0/14)	
4 dpf	0.2% ± 0.2 (0/41, 1/66, 0/77, 0/71, 0/27, 0/32, 0/69)	0% (0/76, 0/67, 0/72)	0%, 0% (0/91, 0/46)	0%, 0% (0/40, 0/41)	0.9% ± 0.6 (1/44, 0/80, 1/81, 0/72)

Notes: Percent mortality was recorded at 5 days post fertilization (dpf). Percent shown is the mean percent mortality ± standard error of the mean when there were more than two repeated experiments. Otherwise, percent from a single trial is listed. Within the parentheses, the ratios of the number of fish that died to the total number of fish are listed, with individual trials separated by commas. DomA= domoic acid, dpf= days post fertilization, SE= standard error of the mean.

Table S5: Acute neurological phenotypes following developmental exposures to domoic acid at 1 dpf

Dose (ng)	Trials	Day observed	Convulsions or pectoral fin flapping mean % ± SE or mean %, mean% (# convulsing/total alive)	No touch response mean % ± SE or mean %, mean% (# no touch response/ total alive)
0	n= 5	2 dpf	0% (0/92, 0/71, 0/64, 0/69, 0/62)	0.4% ± 0.4 (2/92, 0/71, 0/64, 0/69, 0/62)
		3 dpf	0% (0/91, 0/71, 0/64, 0/69, 0/62)	0.2% ± 0.2 (1/91, 0/71, 0/64, 0/69, 0/62)
		4 dpf	0% (0/90, 0/71, 0/64, 0/69, 0/62)	0% (0/90, 0/71, 0/64, 0/69, 0/62)
		5 dpf	0% (0/90, 0/71, 0/64, 0/69, 0/62)	0% (0/90, 0/71, 0/64, 0/69, 0/62)
0.09	n= 2	2 dpf	2.9%, 0% (2/68, 0/69)	32.4%, 15.9% (22/68, 11/69)
		3 dpf	0%, 0% (0/68, 0/68)	0%, 0% (0/68, 0/68)
		4 dpf	0%, 0% (0/68, 0/68)	0%, 0% (0/68, 0/68)
		5 dpf	0%, 0% (0/67, 0/67)	0%, 0% (0/67, 0/67)
0.18	n= 4	2 dpf	8.0% ± 3.0 (5/73, 8/66, 0/75, 4/31)	53.4% ± 12.9 (11/73, 47/66, 47/75, 20/31)
		3 dpf	2.0% ± 1.6 (5/73, 0/66, 1/75, 0/31)	17.3% ± 9.7 (11/73, 4/66, 2/75, 14/31)
		4 dpf	0.8% ± 0.8 (0/73, 0/66, 0/75, 1/31)	0.4% ± 0.4 (1/73, 0/66, 0/75, 0/31)
		5 dpf	0% (0/73, 0/63, 0/75, 0/31)	0% (0/73, 0/63, 0/75, 0/31)

Notes: Percent of fish exhibiting convulsions or pectoral fin flapping (combined) or no touch responses tracked daily (1 day post exposure until 5 days post fertilization (dpf) or between 2-5 dpf). n = the number of repeated experimental trials. Percent shown is the mean percent of fish exhibiting convulsions/fin flapping or no touch responses ± the standard error of the mean if there were more than two repeated trials. Otherwise, values from a single trial are listed. Within the parentheses, the ratios of the number of fish that exhibited convulsions/fin flapping or no touch responses are listed, with individual trials separated by commas. See Figure S3C and Figure S3D for the graphical representation of this table. DomA= domoic acid, dpf= days post fertilization, SE= standard error of the mean.

Table S6: Acute neurological phenotypes following developmental exposures to domoic acid at 2 dpf

Dose (ng)	Trials	Day observed	Convulsions or pectoral fin flapping mean % ± SE or mean %, mean% (# convulsing/total alive)	No touch response mean % ± SE or mean %, mean% (# no touch response/ total alive)
0	n= 11	3 dpf	0% (0/39, 0/71, 0/70, 0/73, 0/58, 0/48, 0/96, 0/20, 0/45, 0/38, 0/58)	0.2% ± 0.2 (1/39, 0/71, 0/70, 0/73, 0/58, 0/48, 0/96, 0/20, 0/45, 0/38, 0/58)
		4 dpf	0% (0/39, 0/71, 0/70, 0/73, 0/58, 0/48, 0/96, 0/20, 0/45, 0/38, 0/58)	0.4% ± 0.3 (1/39, 1/71, 0/70, 0/73, 0/58, 0/48, 0/96, 0/20, 0/45, 0/38, 0/58)
		5dpf	0% (0/39, 0/70, 0/70, 0/73, 0/58, 0/48, 0/96, 0/20, 0/45, 0/38, 0/58)	0.1% ± 0.1 (0/39, 1/70, 0/70, 0/73, 0/58, 0/48, 0/96, 0/20, 0/45, 0/38, 0/58)
0.09	n = 2	3 dpf	2.4%, 0% (2/83, 0/77)	16.9%, 11.7% (14/83, 9/77)
		4 dpf	0%, 0% (0/83, 0/77)	0%, 1.3% (0/83, 1/77)
		5dpf	0%, 0% (0/83, 0/76)	0%, 0% (0/83, 0/76)
0.14	n= 6	3 dpf	23.7% ± 11.6 (4/47, 15/20 10/27, 0/46, 1/36, 9/47)	38.8% ± 10.2 (13/47, 11/20, 22/27, 8/46, 7/36, 15/47)
		4 dpf	0% (0/47, 0/20, 0/27, 0/46, 0/36, 0/47)	1.5% ± 1.5 (0/47, 0/20, 0/27, 4/46, 0/36, 0/47)
		5dpf	0% (0/47, 0/20, 0/27, 0/46, 0/36, 0/47)	0.6% ± 0.6 (0/47, 0/20, 1/27, 0/46, 0/36, 0/47)
0.18	n= 4	3 dpf	40.0% ± 7.4 (27/74, 43/70, 18/66, 25/72)	47.8% ± 16.0 (46/74, 0/70, 41/66, 48/72)
		4 dpf	0.4% ± 0.4 (1/74, 0/53, 0/66, 0/72)	5.6% ± 3.6 (11/74, 4/53, 0/66, 0/72)
		5dpf	1.4% ± 1.4 (0/74, 3/53, 0/60, 0/72)	0.4% ± 0.4 (1/74, 0/53, 0/60, 0/72)

Notes: Percent of fish exhibiting convulsions/pectoral fin flapping or no touch responses tracked daily (1 day post exposure until 5 days post fertilization (dpf) or between 3-5 dpf). n= to the number of repeated experimental trials. Percent shown is the mean percent of fish exhibiting convulsions/fin flapping or no touch responses ± the standard error of the mean if there were more than two repeated trials. Otherwise, values from a single trial are listed. Within the parentheses, the ratios of the number of fish that exhibited convulsions/fin flapping or no touch responses are listed, with individual trials separated by commas. See Figure S3C and Figure S3D for the graphical representation of this table. DomA= domoic acid, dpf= days post fertilization, SE= standard error of the mean.

Table S7: Acute neurological phenotypes following developmental exposures to domoic acid at 4 dpf

Dose (ng)	Trials	Convulsions or pectoral fin flapping mean % ± SE or mean %, mean% (# convulsing/total alive)	No touch response mean % ± SE or mean %, mean% (# no touch response/ total alive)
0 ng	n= 8	0% ± 0 (0/41, 0/65, 0/77 0/71, 0/27, 0/46, 0/32, 0/69)	0% ± 0 (0/41, 0/65, 0/77 0/71, 0/27, 0/46, 0/32, 0/69)
0.09 ng	n= 3	0% ± 0 (0/76, 0/67, 0/72)	0% ± 0 (0/76, 0/67, 0/72)
0.14 ng	n= 2	0%, 0% (0/40, 0/41)	0%, 5.0% (0/41, 2/40)
0.18 ng	n= 4	1.9% ± 1.9 (0/43, 0/80,6/80, 0/72)	24.1% ± 15.3 (0/43, 26/80,51/80, 0/72)

Notes: Percent of fish exhibiting convulsions/fin flapping or no touch responses tracked 1 day post exposure (at 5 dpf). n= to the number of repeated experimental trials. Percent shown is the mean percent of fish exhibiting convulsions/fin flapping or no touch responses ± the standard error of the mean if there were more than two repeated trials. Otherwise, values from a single trial are listed. Within the parentheses, the ratios of the number of fish that exhibited convulsions/fin flapping or no touch responses are listed with individual trials separated by commas. See Figure S3C and Figure S3D for the graphical representation of this table. DomA= domoic acid, dpf= days post fertilization, SE= standard error of the mean.

Table S8: Results from Generalized Estimating Equations (GEE) models assessing the effect of dose and day post-exposure on the prevalence of the lack of touch responses in fish exposed at 1 and 2 days post fertilization

	Exposure at 1 dpf	Exposure at 2 dpf
	Est Coef (Std. error)	**Est Coef (Std. error)**
Intercept	-3.8 (0.5)**	-4.3 (0.4) **
Dose	+22.1 (3.9)**	+24.0 (4.2)**
2 days post exposure	-2.2 (0.7)**	-2.9 (0.6)**
3 days post exposure	-5.8 (1.3)**	-4.9 (0.6)**
4 days post exposure	-46.3 (0.5)**	*NA*

Notes: dpf= days post fertilization, Est Coef= estimated coefficient, Std. error= standard error. Control fish assessed 1 day post exposure was selected as the reference group.** = $p < 0.01$

Table S9: Results from Generalized Estimating Equations (GEE) models assessing the effect of dose and day post-exposure on the prevalence of convulsions or pectoral fin flapping in fish exposed at 1 and 2 days post fertilization

	Exposure at 1 dpf	Exposure at 2 dpf
	Est Coef (Std. error)	**Est Coef (Std. error)**
Intercept	-7.3 (1.2) **	-7.0 (1.1) **
Dose	+26.3 (6.8)**	+37.2 (6.6)**
2 days post exposure	-1.2 (0.8)	-5.4 (0.9)**
3 days post exposure	-3.0 (0.9)**	-4.3 (0.7)**
4 days post exposure	-44.8 (0.8)**	*NA*

Notes: dpf= days post fertilization, Est Coef= estimated coefficient, Std. error= standard error. Control fish assessed 1 day post exposure was selected as the reference group.** **$p < 0.001$, *$p < 0.01$

Table S10: Post-hoc pairwise Dunnett comparisons following binomial modeling of percent responsiveness in startle behavior

	Exposures at 1 dpf		Exposures at 2 dpf		Exposures at 4 dpf	
Dose comparisons (DomA - Control)	**Coef (Std. Er)**	**Pr(>|z|)**	**Coef (Std. Er)**	**Pr(>|z|)**	**Coef (Std. Er)**	**Pr(>|z|)**
0.18 ng – 0 ng == 0	-0.71 (0.13)	**< 1e-4**	-0.64 (0.13)	**2.2e-6**	-0.90 (0.15)	**< 1e- 5**
0.14 ng – 0 ng == 0	-0.77 (0.19)	**0.0003**	-1.10 (0.12)	**< 1e-6**	0.01 (0.28)	1.00
0.13 ng – 0 ng == 0	-0.79 (0.19)	**0.0002**	-0.85 (0.15)	**< 1e-6**	0.10 (0.17)	0.96
0.09 ng – 0 ng == 0	-0.13 (0.15)	0.85	-0.85 (0.14)	**< 1e-6**	0.04 (0.15)	1.00

Notes: Significant values are in **bold red font**. Comparisons were made between fish exposed to different doses of domoic acid (DomA) relative to the control. See Figure 2 for the graphical representation of this table.
DomA= domoic acid, dpf= days post fertilization, Coef= estimated coefficient, Std. Er= standard error, Pr(>|z|)= p-value of the z-statistic.

Table S11: Nonparametric analysis of short latency c-bend startle kinematics following exposure to different doses of domoic acid at 2 days post fertilization

dose (ng)	trial day	n (SLC)	Maximal Angular Velocity (SLC)				Bend angle (SLC)			
			RE	95% CI [Lower, Upper]	Stat	p	RE	95% CI [Lower, Upper]	Stat	p
0.09	5/30/16	0 ng (n= 24), 0.09 ng (n= 40)	0.209	[0.073, 0.346]	-4.877	**2.98e-5**	0.20	[0.068, 0.332]	-5.18	**1.48e-5**
	5/19/16	0 ng (n=37), 0.09 ng (n= 36)	0.115	[0.015, 0.214]	-8.987	**2.56e-10**	0.116	[0.022, 0.211]	-9.1	**1.15e-12**
0.13	5/30/16	0 ng (n= 24), 0.13ng (n= 35)	0.107	[-0.007, 0.221]	-7.877	**1.41e-9**	0.11	[0.004, 0.215]	-8.41	**6.65e-10**
	6/24/16	0 ng (n= 21), 0.13ng (n= 9)	0.001	[0, 0.002]	-1010	**< 1e-14**	0.001	[0, 0.002]	-1010	**< 1e-14**
0.14	6/24/16	0 ng (n= 21), 0.14 ng (n =11)	0.001	[0, 0.002]	-1010	**< 1e-14**	0.001	[0, 0.002]	-1010	**< 1e-14**
	10/10/16	0 ng (n= 34), 0.14 ng (n= 17)	0.112	[0.004, 0.221]	-7.252	**< 1e-14**	0.092	[0, 0.184]	-8.94	**< 1e-14**
	9/23/16	0 ng (n= 42), 0.14 ng (n= 31)	0.06	[-0.006, 0.126]	13.33	**< 1e-14**	0.029	[-0.008, 0.067]	-25.08	**< 1e-14**
0.18	5/19/16	0 ng (n=37), 0.18 ng (n= 26)	0.169	[0.023, 0.316]	-5.253	**1.49e-5**	0.09	[0.002, 0.179]	-10.40	**8.10e-15**
	4/27/16	0 ng (n=17), 0.18 ng (n= 10)	0.071	[-0.056, 0.197]	-7.183	**< 1e-14**	0.094	[-0.039, 0.228]	-6.32	**< 1e-14**
	3/27/16	0 ng (n= 23), 0.18 ng (n=19)	0.005	[-0.007,0.016]	-89.063	**< 1e-14**	0.002	[-0.004, 0.009]	-153.80	**< 1e-14**
	4/15/16	0 ng (n=8), 0.18 ng (n= 8)	0.001	[-0.172, 0.174]	-5.646	**< 1e-14**	0.001	[-0.172, 0.174]	-5.646	**< 1e-14**

Notes: Trials with multiple doses were analyzed using nonparametric multiple comparison procedures with Dunnett-type contrasts to compare kinematics of fish exposed at each dose to the controls (05/30/16, 05/19/16, 06/24/16). Trials with single doses were tested using nonparametric Behrens-Fisher t-tests (10/10/16, 09/23/16, 4/27/16, 03/27/16, 04/15/16). Significant values are in **bold red font**. See Figure 3 for the graphical representation of this table.
SLC= short latency c-bends, DomA= domoic acid, dpf= days post fertilization, RE= estimated relative effect size, Lower= lower limit of the confidence interval, Upper= upper limit of the confidence interval, Stat= test statistic, p= adjusted p-value.

Table S12: Nonparametric analysis of long latency c-bend startle kinematics following exposure to different doses of domoic acid at 2 days post fertilization

dose (ng)	trial day	n (LLC)	Maximal Angular Velocity (LLC)				Bend angle (LLC)			
			RE	95% CI [Lower, Upper]	Stat	p	RE	95% CI [Lower, Upper]	Stat	p
0.09	5/30/16	0 ng (n= 40), 0.09 ng (n= 48)	0.21	[0.099, 0.321]	-5.892	**1.53e-7**	0.238	[0.121, 0.354]	-5.078	**4.51e-6**
	5/19/16	0 ng (n=56), 0.09 ng (n= 47)	0.253	[0.143, 0.363]	-5.027	**4.64e-6**	0.207	[0.105, 0.309]	-6.425	**1.18e-8**
0.13	5/30/16	0 ng (n= 40), 0.13 ng (n= 48)	0.13	[0.046, 0.215]	-9.904	**1.33e-15**	0.161	[0.062, 0.260]	-7.734	**4.19e-11**
	6/24/16	0 ng (n= 24), 0.13 ng (n= 30)	0.044	[-0.025, 0.114]	-14.615	**2.24e-14**	0.062	[-0.019, 0.144]	-12.182	**1.98e-12**
0.14	6/24/16	0 ng (n= 24), 0.14 ng (n = 30)	0.043	[-0.028,0.114]	-14.24	**4.22e-14**	0.054	[-0.021, 0.129]	-13.41	**1.99e-13**
	10/10/16	0 ng (n= 47), 0.14 ng (n= 40)	0.062	[0.005, 0.118]	-15.618	**<1e-14**	0.107	[0.029, 0.185]	-10.078	**<1e-14**
	9/23/16	0 ng (n= 71), 0.14 ng (n= 65)	0.073	[0.028, 0.119]	-18.491	**<1e-14**	0.085	[0.038,0.132]	-17.458	**<1e-14**
0.18	5/19/16	0 ng (n=56), 0.18 ng (n=58)	0.233	[0.127, 0.340]	-5.61	**3.96e-7**	0.201	[0.104, 0.298]	-6.89	**1.41e-9**
	4/27/16	0 ng (n= 19), 0.18 ng (n=20)	0.108	[-0.014, 0.23]	-6.61	**<1e-14**	0.142	[-0.004, 0.288]	-4.976	**<1e-14**
	3/27/16	0 ng (n= 28), 0.18 ng (n = 26)	0.074	[-0.01, 0.159]	-10.298	**<1e-14**	0.11	[0.009, 0.211]	-7.725	**<1e-14**
	4/15/16	0 ng (n=18), 0.18 ng (n= 33)	0.059	[-0.045, 0.162]	-8.966	**<1e-14**	0.072	[-0.042, 0.187]	-7.835	**<1e-14**

Notes: Trials with multiple doses were analyzed using nonparametric multiple comparison procedures with Dunnett-type contrasts to compare kinematics of fish exposed at each dose to the controls (05/30/16, 05/19/16, 06/24/16). Trials with single doses were tested using nonparametric Behrens-Fisher t-tests (10/10/16, 09/23/16, 4/27/16, 03/27/16, 04/15/16). Significant values are in **bold red font**. See Figure 4 for the graphical representation of this table.
LLC= long latency c-bends, DomA= domoic acid, dpf= days post fertilization, RE= estimated relative effect size, Lower= lower limit of the confidence interval, Upper= upper limit of the confidence interval, Stat= test statistic, p= adjusted p-value.

Table S13: Median and Interquartile range for startle kinematic parameters of fish exposed to different doses of domoic acid at 2 dpf

		LLC				SLC			
		Bend angle		Maximal angular velocity		Bend angle		Maximal angular velocity	
Dose (ng)	Trial day	Median	IQR	Median	IQR	Median	IQR	Median	IQR
0	03_27_2016	142.74	28.39	15.36	3.55	139.48	12.51	20.07	1.93
	04_15_2016	134.79	44.27	11.70	4.36	147.88	15.51	19.93	3.71
	04_27_2016	153.34	18.79	15.97	5.05	150.72	17.73	19.03	2.83
	05_19_2016	156.25	63.49	13.27	8.64	156.78	24.74	19.50	1.80
	05_30_2016	143.56	29.35	13.17	6.22	148.01	26.58	19.79	1.83
	06_24_2016	180.46	30.42	17.14	5.14	163.25	15.73	19.72	1.55
	09_23_2016	149.24	35.88	15.32	4.73	150.68	21.75	20.08	1.96
	10_10_2016	155.43	27.64	16.08	5.26	151.30	18.59	20.33	2.53
0.09	05_19_2016	97.4	61.15	8.85	5.99	90.33	60.19	11.51	7.27
	05_30_2016	101.51	76.40	7.24	5.78	102.85	78.20	10.59	12.63
0.13	05_30_2016	76.57	74.10	4.94	5.90	74.69	51.29	10.33	9.42
	06_24_2016	83.02	55.10	5.67	3.01	49.15	36.17	5.09	2.13
0.14	06_24_2016	95.69	46.50	6.13	3.47	64.37	29.90	6.27	2.87
	09_23_2016	78.58	45.61	5.8	4.01	58.06	44.90	5.73	3.53
	10_10_2016	81.29	66.03	6.3	4.29	46.50	56.58	5.69	3.11
0.18	03_27_2016	75.02	63.27	5.68	4.55	56.61	61.86	6.66	7.27
	04_15_2016	59.56	39.59	4.26	2.41	37.12	17.42	4.59	2.90
	04_27_2016	49.77	59.17	4.00	2.52	54.28	42.04	6.98	4.98
	05_19_2016	71.3	58.68	6.60	3.97	67.48	79.20	6.38	10.59

Note: IQR= interquartile range, 3rd quantile- 1st quantile, LLC= long latency c-bends, SLC= short latency c-bends. This table supports Figures 3 and 4.

Table S14: Nonparametric analysis of short latency c-bend startle kinematics following exposure to different doses of domoic acid at 1 day post fertilization

dose (ng)	trial day	n (SLC)	Maximal Angular Velocity (SLC)				Bend angle (SLC)			
			RE	95% CI [Lower, Upper]	Stat	p	RE	95% CI [Lower, Upper]	Stat	p
0.09	5/30/16	0 ng (n= 39), 0.09 ng (n= 36)	0.555	[0.391, 0.719]	0.800	0.669	0.426	[0.268, 0.584]	-1.10	0.469
	5/19/16	0 ng (n=25), 0.09 ng (n= 25)	0.419	[0.226, 0.613]	-0.96	0.532	0.581	[0.387, 0.774]	0.96	0.536
0.13	5/30/16	0 ng (n= 39), 0.13 ng (n=19)	0.356	[0.140, 0.573]	-1.59	0.232	0.309	[0.121, 0.497]	-2.39	**0.045**
0.14	9/04/16	0 ng (n=35), 0.14 ng(n=35)	0.557	[0.416, 0.697]	0.81	0.423	0.347	[0.21,0.484]	-2.24	**0.029**
0.18	5/19/16	0 ng (n=25), 0.18 ng (n=18)	0.449	[0.235,0.663]	-0.55	0.805	0.549	[0.336, 0.762]	0.53	0.82
	4/27/16	0 ng (n=13), 0.18 ng (n= 16)	0.279	[0.078, 0.48]	-2.26	**0.032**	0.274	[0.077,0.471]	-2.36	**0.026**
	4/15/16	0 ng (n=12), 0.18 ng (n= 12)	0.34	[0.088, 0.593]	-1.32	0.203	0.222	[0.01, 0.435]	-2.71	**0.013**

Notes: Trials with multiple doses were analyzed using nonparametric multiple comparison procedures with Dunnett-type contrasts to compare kinematics of fish exposed at each dose to the controls (05/30/16, 05/19/16). Trials with single doses were tested using nonparametric Behrens-Fisher t-tests (09/04/16, 04/27/16, 04/15/16). Significant values are in **bold red font**. See Figure 3 for the graphical representation of this table.
SLC= short latency c-bends, DomA= domoic acid, dpf= days post fertilization, RE= estimated relative effect size, Lower= lower limit of the confidence interval, Upper= upper limit of the confidence interval, Stat= test statistic, p= adjusted p-value.

Table S15: Nonparametric analysis of long latency c-bend startle kinematics following exposure to different doses of domoic acid at 1 day post fertilization

			Maximal Angular Velocity (LLC)				Bend angle (LLC)			
dose (ng)	**trial day**	**n (LLC)**	**RE**	**95% CI [Lower, Upper]**	**Stat**	**p**	**RE**	**95% CI [Lower, Upper]**	**Stat**	**p**
0.09	5/30/16	0 ng (n= 47), 0.09 ng (n= 51)	0.429	[0.296, 0.562]	-1.212	0.39	0.476	[0.340, 0.613]	-0.398	0.901
	5/19/16	0 ng (n= 37), 0.09 ng (n= 35)	0.492	[0.334, 0.650]	-0.115	0.99	0.544	[0.388, 0.699]	0.6313	0.742
0.13	5/30/16	0 ng (n= 47), 0.13 ng (n= 31)	0.298	[0.156, 0.440]	-3.24	**0.004**	0.321	[0.163, 0.478]	-2.63	**0.023**
0.14	9/04/16	0 ng (n=50), 0.14 ng (n= 48)	0.385	[0.271, 0.498]	-2.02	**0.046**	0.335	[0.224, 0.447]	-2.941	**0.004**
0.18	5/19/16	0 ng (n= 37), 0.18 ng (n= 41)	0.372	[0.228, 0.517]	-1.984	0.09	0.405	[0.258, 0.552]	-1.454	0.249
	4/27/16	0 ng (n= 15), 0.18 ng (n= 17)	0.118	[-0.012, 0.247]	-6.087	**< 1e-14**	0.11	[-0.004, 0.224]	-6.997	**< 1e-14**
	4/15/16	0 ng (n= 36), 0.18 ng (n= 37)	0.354	[0.221, 0.486]	-2.214	**0.031**	0.322	[0.196, 0.448]	-2.817	**0.006**

Notes: Trials with multiple doses were analyzed using nonparametric multiple comparison procedures with Dunnett-type contrasts to compare kinematics of fish exposed at each dose to the controls (05/30/16, 05/19/16). Trials with single doses were tested using nonparametric Behrens-Fisher t-tests (09/04/16, 04/27/16, 04/15/16). Significant values are in **bold red font**. See Figure 4 for the graphical representation of this table.
LLC= long latency c-bends, DomA= domoic acid, dpf= days post fertilization, RE= estimated relative effect size, Lower= lower limit of the confidence interval, Upper= upper limit of the confidence interval, Stat= test statistic, p= adjusted p-value.

Table S16: Nonparametric analysis of short latency c-bends kinematics following exposure to different doses of domoic acid at 4 days post fertilization

			Maximal Angular Velocity (SLC)				Bend angle (SLC)			
dose (ng)	**trial day**	**n (SLC)**	**RE**	**95% CI [Lower, Upper]**	**Stat**	**p**	**RE**	**95% CI [Lower, Upper]**	**Stat**	**p**
0.09	5/30/16	0 ng (n= 31), 0.09 ng (n= 48)	0.544	[0.390, 0.697]	0.642	0.741	0.545	[0.394, 0.696]	0.676	0.730
	5/19/16	0 ng (n=20), 0.09 ng (n = 17)	0.435	[0.181, 0.690]	-0.624	0.781	0.435	[0.196, 0.675]	-0.654	0.756
0.13	5/30/16	0 ng (n= 31), 0.13 ng (n= 45)	0.466	[0.315, 0.617]	-0.509	0.826	0.448	[0.296, 0.600]	-0.776	0.662
	6/24/16	0 ng (n= 5), 0.13 ng (n = 11)	0.618	[0.240, 0.996]	0.778	0.68	0.600	[0.214, 0.986]	0.651	0.750
0.14	6/24/16	0 ng (n= 5), 0.14 ng(n= 13)	0.585	[0.239, 0.930]	0.61	0.792	0.338	[-0.009,-0.686]	-1.167	0.429
0.18	5/19/16	0 ng (n=20), 0.18 ng (n= 10)	0.430	[0.138, 0.722]	-0.588	0.802	0.37	[0.097, 0.643]	-1.15	0.441
	4/27/16	0 ng (n= 13), 0.18 ng (n= 8)	0.212	[-0.058, 0.481]	-2.309	**0.038**	0.183	[-0.026, 0.391]	-3.185	**0.005**
	3/27/16	0 ng (n=19), 0.18 ng (n= 10)	0.363	[0.107, 0.62]	-1.143	0.272	0.389	[0.15, 0.629]	-0.967	0.346

Notes: Trials with multiple doses were analyzed using nonparametric multiple comparison procedures with Dunnett-type contrasts to compare kinematics of fish exposed at each dose to the controls (05/30/16, 05/19/16, 06/24/16). Trials with single doses were tested using nonparametric Behrens-Fisher t-tests (04/27/16, 03/27/16). Significant values are in **bold red font**. See Figure 3 for the graphical representation of this table.

SLC= short latency c-bends, DomA= domoic acid, dpf= days post fertilization, RE= estimated relative effect size, Lower= lower limit of the confidence interval, Upper= upper limit of the confidence interval, Stat= test statistic, p= adjusted p-value.

Table S17: Nonparametric analysis of long latency c-bends kinematics following exposure to different doses of domoic acid at 4 days post fertilization

			Maximal Angular Velocity (LLC)				Bend angle (LLC)			
dose (ng)	**trial day**	**n (LLC)**	**RE**	**95% CI [Lower, Upper]**	**Stat**	**p**	**RE**	**95% CI [Lower, Upper]**	**Stat**	**p**
0.09	5/30/16	0 ng (n= 37), 0.09 ng (n= 45)	0.499	[0.353, 0.645]	-0.014	1.000	0.546	[0.399, 0.693]	0.706	0.713
	5/19/16	0 ng (n= 47), 0.09 ng (n= 36)	0.592	[0.442, 0.741]	1.441	0.289	0.472	[0.321, 0.623]	-0.430	0.887
0.13	5/30/16	0 ng (n= 37), 0.13 ng (n= 47)	0.511	[0.365, 0.657]	0.173	0.978	0.56	[0.413, 0.706]	0.921	0.568
	6/24/16	0 ng (n=18), 0.13 ng (n = 33)	0.283	[0.102, 0.464]	-2.763	**0.017**	0.226	[0.059, 0.392]	-3.809	**0.001**
0.14	6/24/16	0 ng (n=18), 0.14 ng (n= 22)	0.328	[0.123, 0.533]	-1.93	0.1089	0.283	[0.085, 0.481]	-2.529	**0.030**
0.18	5/19/16	0 ng (n= 47), 0.18 ng (n= 26)	0.394	[0.203, 0.585]	-1.309	0.355	0.337	[0.163, 0.511]	-2.184	0.069
	4/27/16	0 ng (n= 11), 0.18 ng (n= 11)	0.504	[0.215, 0.793]	0.03	0.976	0.413	[0.144, 0.682]	-0.676	0.507
	3/27/16	0 ng (n=14), 0.18 ng (n= 12)	0.482	[0.238,0.726]	-0.151	0.881	0.411	[0.171, 0.65]	-0.769	0.449

Notes: Trials with multiple doses were analyzed using nonparametric multiple comparison procedures with Dunnett-type contrasts to compare kinematics of fish exposed at each dose to the controls (05/30/16, 05/19/16, 06/24/16). Trials with single doses were tested using nonparametric Behrens-Fisher t-tests (04/27/16, 03/27/16). Significant values are in **bold red font**. See Figure 4 for the graphical representation of this table.

LLC= long latency c-bends, DomA= domoic acid, dpf= days post fertilization, RE= estimated relative effect size, Lower= lower limit of the confidence interval, Upper= upper limit of the confidence interval, Stat= test statistic, p= adjusted p-value.

Table S18: Nonparametric analysis of long latency c-bends kinematics in DomA-exposed larvae with or without inflated swim bladders

		Maximal Angular Velocity (LLC)				Bend angle (LLC)			
treatment	**n (LLC)**	**RE**	**95% CI [Lower, Upper]**	**Stat**	**p**	**RE**	**95% CI [Lower, Upper]**	**Stat**	**p**
DomA +SB	Control +SB (n= 147), DomA +SB (n= 42)	0.094	[0.038, 0.151]	-18.74	**<1 e-6**	0.107	[0.036, 0.178]	-14.51	**1.69 e-13**
DomA -SB	Control +SB (n= 147), DomA -SB (n= 77)	0.042	[0.004, 0.079]	-31.74	**<1 e-6**	0.071	[0.021, 0.120]	-22.81	**<1 e-6**
Control -SB	Control +SB (n= 147), Control -SB (n= 17)	0.146	[0.004, 0.288]	-6.46	**9.7 e-6**	0.174	[0.005, 0.344]	-5.02	**2.49 e-4**

Notes: Nonparametric multiple comparison procedures with Dunnett-type contrasts were employed to compare kinematics of DomA-exposed fish with different morphological attributes (with or without swim bladders) to the control fish with swim bladders. A further comparison is made with control fish swim bladders to control fish without swim bladders. Significant values are in **bold red font**. See Figure S5A and Figure S5B for the graphical representation of the data.
DomA= domoic acid, SB= swim bladder, LLC = long latency c-bends, RE= estimated relative effect size, Lower= lower limit of the confidence interval, Upper= upper limit of the confidence interval, Stat= test statistic, p= adjusted p-value.

Table S19: Nonparametric analysis of long latency c-bends kinematics in DomA-exposed larvae without bent body axes or with bent body axes

		Maximal Angular Velocity (LLC)				Bend angle (LLC)			
treatment	**n (LLC)**	**RE**	**95% CI [Lower, Upper]**	**Stat**	**p**	**RE**	**95% CI [Lower, Upper]**	**Stat**	**p**
DomA no bent	Control no bend (n= 154), DomA no bend (n= 71)	0.085	[0.033, 0.136]	-22.5	**1.23 e-14**	0.102	[0.042, 0.162]	-18.62	**3.92 e-11**
DomA with bent	Control no bend (n= 154), DomA with bend (n= 48)	0.053	[-0.002, 0.109]	-22.66	**<1 e-16**	0.082	[0.009, 0.155]	-16.22	**6.58 e-11**
Control with bent	Control no bend (n= 154), Control with bend (n= 10)	0.135	[-0.075, 0.345]	-4.87	**1.81 e 03**	0.123	[-0.040, 0.286]	-6.52	**1.51 e-4**

Notes: Nonparametric multiple comparison procedures with Dunnett-type contrasts were employed to compare kinematics of DomA-exposed fish with different morphological attributes (without a bent body axis or with a bent body axis) to the control fish without a bent body axis. A further comparison is made with control fish without a bent body axis to control fish with a bent body axis. Significant values are in **bold red font**. See Figure S5C and Figure S5D for the graphical representation of the data.

DomA= domoic acid, with bend= bent body axis, no bend= straight body axis, LLC = long latency c-bends, RE= estimated relative effect size, Lower= lower limit of the confidence interval, Upper= upper limit of the confidence interval, Stat= test statistic, p= adjusted p-value.

Table S20: Myelin phenotype classification by day injected using confocal microscopy

	Control						Domoic acid					
		Myelin category						Myelin category				
Day injected	**total**	**0**	**1**	**2**	**3**	**4**	**total**	**0**	**1**	**2**	**3**	**4**
1 dpf	22 (15, 7)	22	0	0	0	0	31 (25, 6)	31	0	0	0	0
1.5 dpf	24 (18, 6)	24	0	0	0	0	34 (23, 11)	23	3	3	5	0
2 dpf	61 (7, 8, 24, 22)	61	0	0	0	0	106 (31, 32, 36, 7)	10	19	45	29	3
2.5 dpf	29 (21, 8)	29	0	0	0	0	40 (33, 7)	5	9	20	5	1
4 dpf	40 (6, 10, 24)	40	0	0	0	0	46 (14, 24, 8)	44	1	0	1	0

Notes: Fish were exposed to domoic acid (DomA) at a nominal dose that ranged from 0.13- 0.14 ng, which was administered at a specified developmental time period in days post fertilization (dpf). Fish were then imaged at 5 dpf. Control counterparts were exposed to vehicle saline. The total column has two rows: the first row lists the total number of fish imaged from all the repeated experimental trials, and the second row contains numbers in parentheses that include the total number of fish imaged in each experimental trial, with each trial separated by commas. Fish were also classified into different myelin categories (0- 4). A description of myelin categories can be found in Figure S2. See Figure 5 for the graphical representation of this table.

Table S21: Trials included to assess myelin labeling, imaged using widefield epifluorescence microscopy at 5 days post fertilization (dpf)

		Control	DomA			
		0 ng	**0.09 ng**	**0.13 ng**	**0.14 ng**	**0.18 ng**
Day injected	**1 dpf**	n=24, 12, 6, 18, 64, 37	n=25, 16, 11	n=6	n= 68, 40	n=28, 51,15
	1.5 dpf	n= 30	-	-	-	n= 48
	2 dpf	n= 23, 37, 29, 19, 29, 70, 82, 80, 71, 30, 21, 80, 58	n=51, 31, 13	n=12, 34, 43	n=35, 68, 75, 77, 81, 40, 24, 80, 49	n= 26, 59, 27, 41
	2.5 dpf	n= 18, 55, 17	-	-	n= 20, 62, 36	-
	3 dpf	n = 6, 52, 45	-	n= 12	n= 8, 77, 36	-
	4 dpf	n= 14, 17, 18, 19, 50, 24	n=17, 10	n= 10, 32	n= 19, 27, 48, 21, 77	-

Note: n corresponds to the number of fish within a trial that were exposed to a given dose of domoic acid (DomA) administered at the specified developmental time period in dpf (days post fertilization). This table supports Figure 6.

Table S22: Myelin phenotype classification by dose and day injected after fish were imaged using widefield epifluorescence microscopy at 5 days post fertilization

Day injected (dpf)	0 ng							0.09 ng							0.13 ng							0.14 ng							0.18 ng						
		Myelin category							Myelin category							Myelin category							Myelin category							Myelin category						
	tot	0	1	2	3	4	5	tot	0	1	2	3	4	5	tot	0	1	2	3	4	5	tot	0	1	2	3	4	5	tot	0	1	2	3	4	5
1	161	160	0	1	0	0	0	52	52	0	0	0	0	0	6	5	1	0	0	0	0	108	103	1	3	0	1	0	94	83	0	9	0	0	2
1.5	30	30	0	0	0	0	0																						48	38	3	7	0	0	0
2	629	624	3	1	1	0	0	95	9	30	14	28	14	0	89	0	16	13	52	7	1	529	17	110	75	268	59	0	153	4	30	14	64	41	0
2.5	90	90	0	0	0	0	0															118	20	17	35	41	3	2							
3	103	102	0	0	0	0	1								12	5	0	2	2	2	1	121	76	2	17	8	2	16							
4	142	141	0	0	1	0	0	27	26	1	0	0	0	0	42	42	0	0	0	0	0	192	190	0	0	0	0	2							

Note: Numbers correspond to the number of fish in each myelin category (0-5). Total (tot) reflects the sum of fish across all categories. A description of myelin categories can be found in Figure S2. See Figure 6 for the graphical representation of this table.
dpf= days post fertlization, tot = total number of fish imaged.

Table S23: Drop-in-deviance test for incorporating day of exposure into multinomial logistic regression model

Model number	Model	Residual df	Residual deviance	Test	Df	LR stat.	Pr (Chi)
1	$\beta_0 + \beta_{dose}$	14180	5064.531		NA	NA	NA
2	$\beta_0 + \beta_{dose} + \beta_{DayExposure}$	14170	3418.670	1 vs. 2	10	1645.86	<1 e-6

Notes: Df= degrees of freedom, LR Stat. = Likelihood-ratio statistic, Pr (Chi)= P values from Chi squared test

Table S24: Multinomial logistic regression model for distribution of myelin phenotypes in fish exposed to 0.14 ng of domoic acid at different periods in development (in days post fertilization)

Myelin category	Intercept	2 dpf inj	2.5 dpf inj	3 dpf inj	4 dpf inj
1	-4.63 (p= 3.98e-6)	6.50 (p= 3.76e-10)	4.47 (p= 2.35e-5)	1.00 (p= 4.19e-1)	-9.42 (p= 0.91)
2	-3.54 (p= 1.56e-9)	5.02 (p= 6.66e-15)	4.10 (p= 2.83e-10)	2.04 (p= 1.55e-3)	-19.65 (p< e-16)
3	-31.83 (p= 7.00e-11)	34.58 (p= 1.44e-12)	32.54 (p= 2.71e-11)	29.57 (p= 1.46e-9)	20.19 (p= 0.30)
4	-4.64 (p= 4.00e-6)	5.88 (p= 1.68e-8)	2.74 (p= 2.04e-2)	1.00 (p= 4.19e-1)	-7.74 (p= 0.83)
5	-31.01 (p< e-16)	11.57 (p< e-16)	28.70 (p< e-16)	29.45 (p< e -16)	26.45 (p< e-16)

Note: The reference condition for the analysis is 1 dpf injected fish with no myelin deficits (myelin category 0). A description of myelin categories can be found in Figure S2. Coefficients are listed along with p-values in parentheses. See Figure 6 for the graphical representation of this table.
DomA = domoic acid, dpf= days post fertilization

Table S25: Trials included to assess myelin labeling, imaged using widefield epifluorescence microscopy at 6 days post fertilization

	Control	DomA		
Day injected	**0 ng**	**0.09 ng**	**0.13 ng**	**0.18 ng**
1 dpf	n= 27, 10, 32	n= 22, 36	n= 36	n= 25, 14
2 dpf	n= 22, 15	n= 52	n= 41	-
4 dpf	n= 31, 24, 34	n= 35, 13, 41	n= 37	-

Note: n corresponds to the number of fish within a trial that were exposed to a given dose of domoic acid (DomA) administered at the specified developmental time period in days post fertilization (dpf). This table supports Figure 7.

Table S26: Myelin phenotype classification by dose and day injected after fish were imaged using widefield epifluorescence microscopy at 6 days post fertilization

Day injected (dpf)	0 ng							0.09 ng							0.13 ng							0.18 ng						
		Myelin category							Myelin category							Myelin category							Myelin category						
	tot	0	1	2	3	4	5	*tot*	0	1	2	3	4	5	*tot*	0	1	2	3	4	5	*tot*	0	1	2	3	4	5
1	*69*	69	0	0	0	0	0	*58*	58	0	0	0	0	0	*36*	34	2	0	0	0	0	*39*	32	1	0	0	0	6
2	*37*	37	0	0	0	0	0	*52*	13	18	14	5	2	0	*41*	8	14	7	11	1	0							
4	*89*	88	0	1	0	0	0	*89*	88	1	0	0	0	0	*37*	36	1	0	0	0	0							

Note: Numbers correspond to the number of fish in each myelin category (0-5). Total (Tot) reflects the sum of fish across all categories. A description of myelin categories can be found in Figure S2. See Figure 7A for the graphical representation of this table.
dpf= days post fertilization, tot = total number of fish imaged.

Table S27: Trials included to assess myelin labeling, imaged using widefield epifluorescence microscopy at 7 days post fertilization

	Control	DomA			
Day injected	**0 ng**	**0.09 ng**	**0.13 ng**	**0.14 ng**	**0.18 ng**
1 dpf	n=23	n= 18	n=17	-	-
2 dpf	n=14, 10, 2, 28	n=18	n=19	n=39	n=39, 19, 21
4 dpf	n=21, 16	n=19	n=20	n=25	-

Note: n corresponds to the number of fish within a trial that were exposed to a given dose of domoic acid (DomA) administered at the specified developmental time period. See Figure 7B for the graphical representation of this table.

Table S28: Myelin phenotype classification by domoic acid dose and day injected after fish were imaged using widefield epifluorescence microscopy at 7 days post fertilization

Day injected (dpf)	0 ng							0.09 ng							0.13 ng							0.14 ng							0.18 ng						
		Myelin category							Myelin category							Myelin category							Myelin category							Myelin category						
	tot	**0**	**1**	**2**	**3**	**4**	**5**	*tot*	**0**	**1**	**2**	**3**	**4**	**5**	*tot*	**0**	**1**	**2**	**3**	**4**	**5**	*tot*	**0**	**1**	**2**	**3**	**4**	**5**	*tot*	**0**	**1**	**2**	**3**	**4**	**5**
1	*23*	23	0	0	0	0	0	*18*	18	0	0	0	0	0	*17*	17	0	0	0	0	0														
2	*54*	51	3	0	0	0	0	*18*	3	9	3	3	0	0	*19*	1	6	2	10	0	0	*39*	3	15	15	6	0	0	*40*	1	13	7	16	3	0
4	*37*	37	0	0	0	0	0	*19*	19	0	0	0	0	0	*20*	20	0	0	0	0	0	*25*	25	0	0	0	0	0							

Note: Numbers correspond to the number of fish in each myelin category (0-5). Total (Tot) reflects the sum of fish across all categories. A description of myelin categories can be found in Figure S2. See Figure 7B for the graphical representation of this table.
dpf= days post fertilization, tot = total number of fish imaged.

Table S29: Multinomial logistic regression model for distribution of myelin phenotypes in fish exposed to different doses of domoic acid at 2 days post fertilization (dpf) and imaged at 5, 6, and 7 dpf

myelin category	5 dpf imaged		6 dpf imaged		7 dpf imaged	
	Intercept	DomA (ng)	Intercept	DomA (ng)	Intercept	DomA (ng)
1	-4.85 (p <e-16)	52.46 (p <e-16)	-3.82 (p =2.04 e -5)	40.21 (p =6.73 e -6)	-2.77 (p =1.54e-7)	34.12 (p =7.37e-11)
2	-5.49 (p <e-16)	53.64 (p <e-16)	-3.66 (p =3.52 e -5)	34.72 (p =9.2 e-5)	-4.42 (p =1.34e-6)	42.15 (p =1.02e-8)
3	-5.06 (p <e-16)	59.56 (p <e-16)	-6.79 (p =7.88 e -5)	60.37 (p =7.87 e-5)	-4.96 (p =5.93e-7)	47.74 (p =8.54e-10)
4	-7.73 (p <e-16)	69.59 (p <e-16)	-5.59 (p =8.96 e -3)	34.54 (p =0.087)	-15.46 (p =2.64e-2)	96.52 (p =1.61e-2)
5	-9.09 (p =0.005)	44.81 (p =0.06)	NA	NA	NA	NA

Notes: Domoic acid (DomA) is modeled as a continuous factor from fish exposed to the following nominal DomA doses: 0.09, 0.13, and 0.14 ng. Coefficients were listed along with p-values in parentheses. See Figure 6 (5 dpf imaged), Figure 7A (6 dpf imaged), and Figure 7B (7 dpf imaged) for the graphical representations of this table.

Table S30: Genes associated with the enriched GO term: biological processes

GO term	term ID	pvalue	ENSEMBL_gene	gene name	logFC
Protein depolymerization	GO:0051261	3.54E-02	ENSDARG00000030106	*stmn4*	0.507
Microtubule depolymerization	GO:0007019	8.71E-03	ENSDARG00000038465	*stmn3*	-0.612
			ENSDARG00000043932	*stmn4l*	0.433

Notes: Genes associated with the GO term are listed by ENSEMBL gene ID and common gene name. log fold change (FC) is also indicated. Functional enrichment analysis was done using gprofiler (version r1750_e91_eg38), which employs a hypergeometric test (Fisher's exact test) with a multiple testing correction using the g:SCS threshold. See Figure 9C for the graphical representation of this table.

Table S31: Human Phenotype Ontology associated with differentially expressed genes at 3 days post fertilization

Human phenology phenotype	Term ID	pvalue	ENSDARG00000012426 *neflb*	ENSDARG00000057568 *nefla*	ENSDARG00000038609 *mpz*	ENSDARG00000039522 *tubb2*	ENSDARG00000018997 *cplx2l*
Peripheral axonal degeneration	HP:0000764	2.12 e-2	■	■	■	■	
Myelin outfoldings	HP:0004336	4.50 e-4	■	■	■		
Peripheral hyphermyelination	HP:0030173	7.85 e-4	■	■	■		
Segmental peripheral demyelination/ remyelination	HP:0003481	8.06 e-3	■	■	■		
Clusters of axonal regeneration	HP:0007233	8.23 e-3	■	■			
Ulnar claw	HP:0001178	3.68 e-3	■	■	■		
Hypotrophy of small hand muscles	HP:0006006	4.92 e-2	■	■	■		
Ectrodactyly	HP:0100257	4.37 e-2	■	■	■		■
Split hand	HP:0001171	3.89 e-2	■	■	■		■

Notes: Genes associated with each Human Phenotype Ontology are listed by ENSEMBL gene ID and common gene name. Shading indicates which of the listed genes are associated with each phenotype. Functional enrichment analysis was done using gprofiler (version r1750_e91_eg38), which employs a hypergeometric test (Fisher's exact test) with a multiple testing correction using the g:SCS threshold. See Figure 9C and Figure 10 for the graphical representations of this table.

Video S1: Acoustic startle response

A 16 well plate containing control fish in the top two rows and domoic acid (DomA)-exposed fish (2 dpf injected, 0.14ng) in the bottom two rows. Fish were subjected to an auditory/vibrational stimulus, while their responses were recorded at 1000 frames per second.

Video S2: Time-lapse video of *Tg(sox10:RFP)* x *Tg(nkx2.2a:mEGFP)* control fish

Time-lapse sequence of the spinal cord of a control fish injected at 2 days post fertilization (dpf) taken from 2.5- 3 dpf (12.5 hours in total, with a 9 minute imaging interval). RFP labels cell bodies of cells from the oligodendrocyte lineage. mEGFP expression labels oligodendrocyte membrane processes that wrap axons and become elongated, nascent myelin sheaths.

Video S3: Time-lapse video of *Tg(sox10:RFP)* x *Tg(nkx2.2a:mEGFP)* DomA exposed fish

Time-lapse sequence of the spinal cord of a domoic acid (DomA)-exposed fish (0.14 ng) injected at 2 dpf taken from 2.5- 3 dpf (12.5 hours in total, with a 9 minute imaging interval). RFP labels cell bodies of cells from the oligodendrocyte lineage. mEGFP expression labels oligodendrocyte membrane processes that form unusual circular membranes.

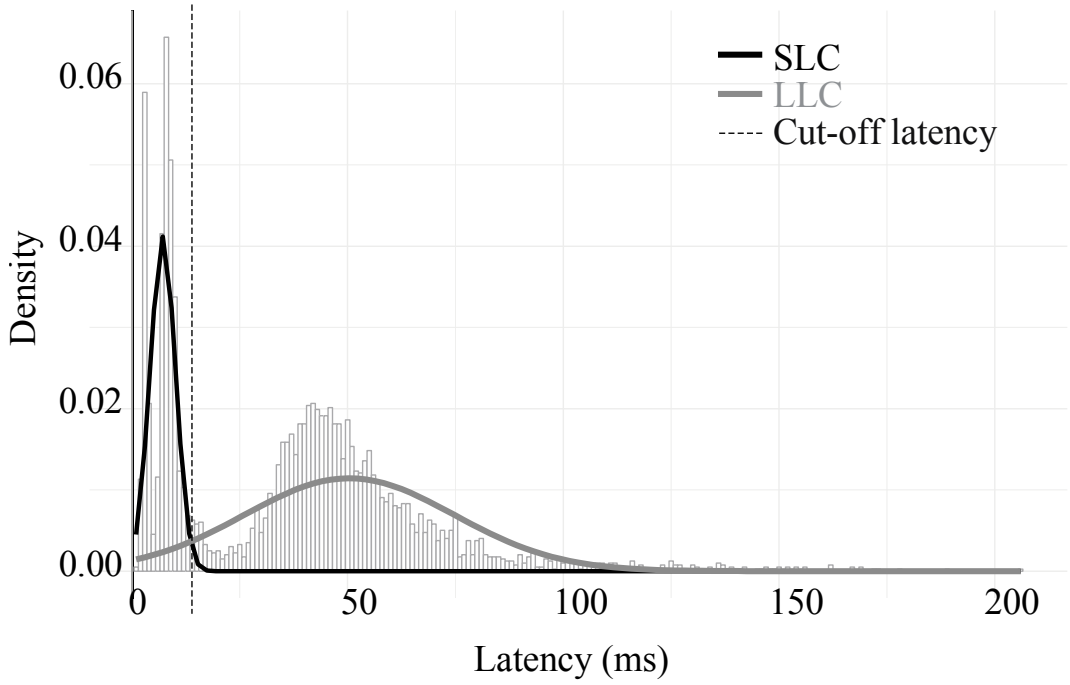


Figure S1: Startle behavioral classification

Density histogram of the latency distribution for control fish. Overlaid are two Gaussian curves that were fit to the data. The black curve represents the Short latency c-bend (SLC) startle response distribution, and gray curve represents the Long latency c-bend (LLC) startle response distribution. The dashed black vertical line at 13 milliseconds represents the cut-off by which there is a greater than 50% probability of a given data point belonging to either modeled distribution.

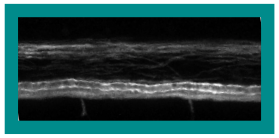
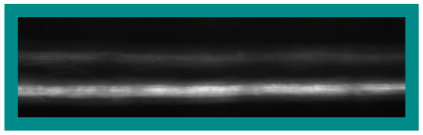
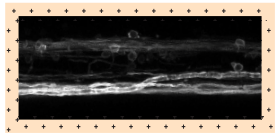
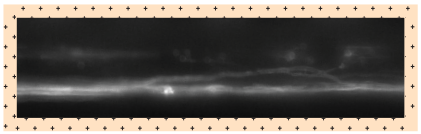
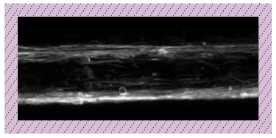
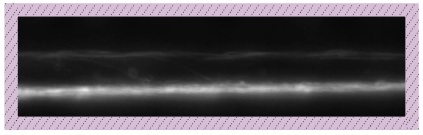
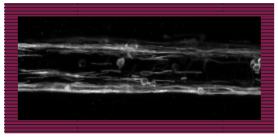
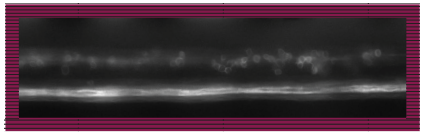
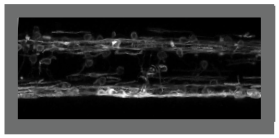
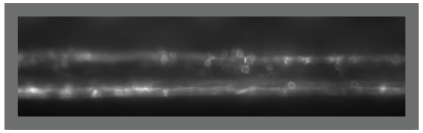
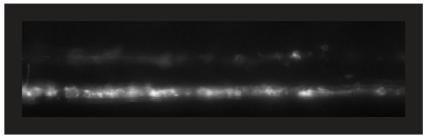
Sample confocal microscopy images	Sample widefield microscopy images	Severity Classification (number) - Description
		(0) Normal phenotype — Dorsal and ventral regions of the spinal cord had labeled myelin sheaths. The myelin sheath surrounding the Mauthner axon was visible.
		(1) Myelin sheaths were present but disorganized. In some cases, myelinated axons that were normally found ventrally were located more dorsally. In others, the myelinated axons terminated prematurely with distal ends located more dorsally.
		(2) Myelin was labeled in both the dorsal and ventral regions of the spinal cord, but there are some noticeable deficits. While the ventral spinal cord was labeled, it had noticeably less myelin labeled compared to controls.
		(3) The loss of labeled myelin in the ventral spinal cord resulted in large, observable gaps between myelinated axons.
		(4) Myelin sheaths were essentially nonexistent in the ventral spinal cord. Instead, numerous hollow circular profiles were present both in the ventral and dorsal spinal cord.
		(5) There were visible sloughed off portions of myelin which were defined by labeled portions of myelin which had a ‘rough looking appearance’ and were separated from the thin, elongated sheaths

Figure S2: Qualitative myelin phenotype scoring

Myelinated axonal tracks in the dorsal and ventral spinal cord. Fish were blindly classified into 6 categories (0-5) based on the severity in the myelin defect. Representative confocal and widefield fluorescence microscopy images are shown for each severity classification.

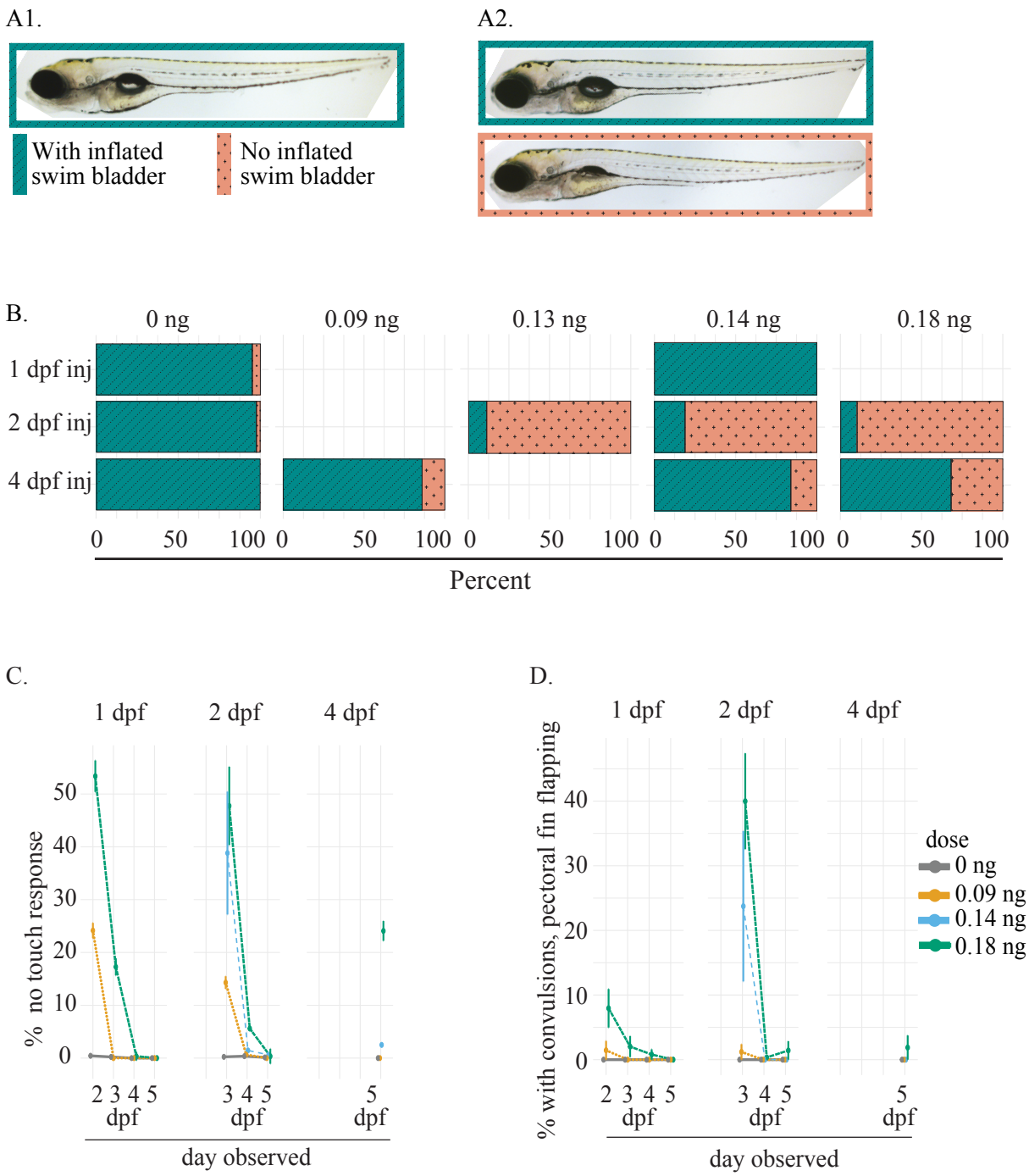


Figure S3: Acute neurotoxic and morphological phenotypes associated with developmental exposure to domoic acid

(A1) Representative brightfield image of control larvae with an inflated swim bladder.

(A2) Representative brightfield image of domoic acid (DomA)-exposed larvae with an inflated swim bladder (teal), and without an inflated swim bladder (peach).

(B) Presence or absence of the inflated swim bladder was determined by treatment, dose, and time of exposure. (See also Table S2.)

(C) Fish were exposed to different doses of DomA (0.09 ng- 0.18 ng) at 1, 2, and 4 dpf. Within individual trials, the percentage of embryos with touch response deficits were recorded one day post-exposure until 5 dpf. Points represent the mean prevalence of no touch responses from multiple repeated trials ± standard error (SE).

(D) The same fish population observed in Figure S3C were also monitored for the presence of convulsions or pectoral fin flapping from one day post-exposure until 5 dpf.

Statistical significance in **(C)** and **(D)** was determined using generalized estimating equations (for 1 and 2 dpf injected fish), or using a generalized linear model with a quasibinomial link function (for 4 dpf injected fish) (see also Table S5-S9).

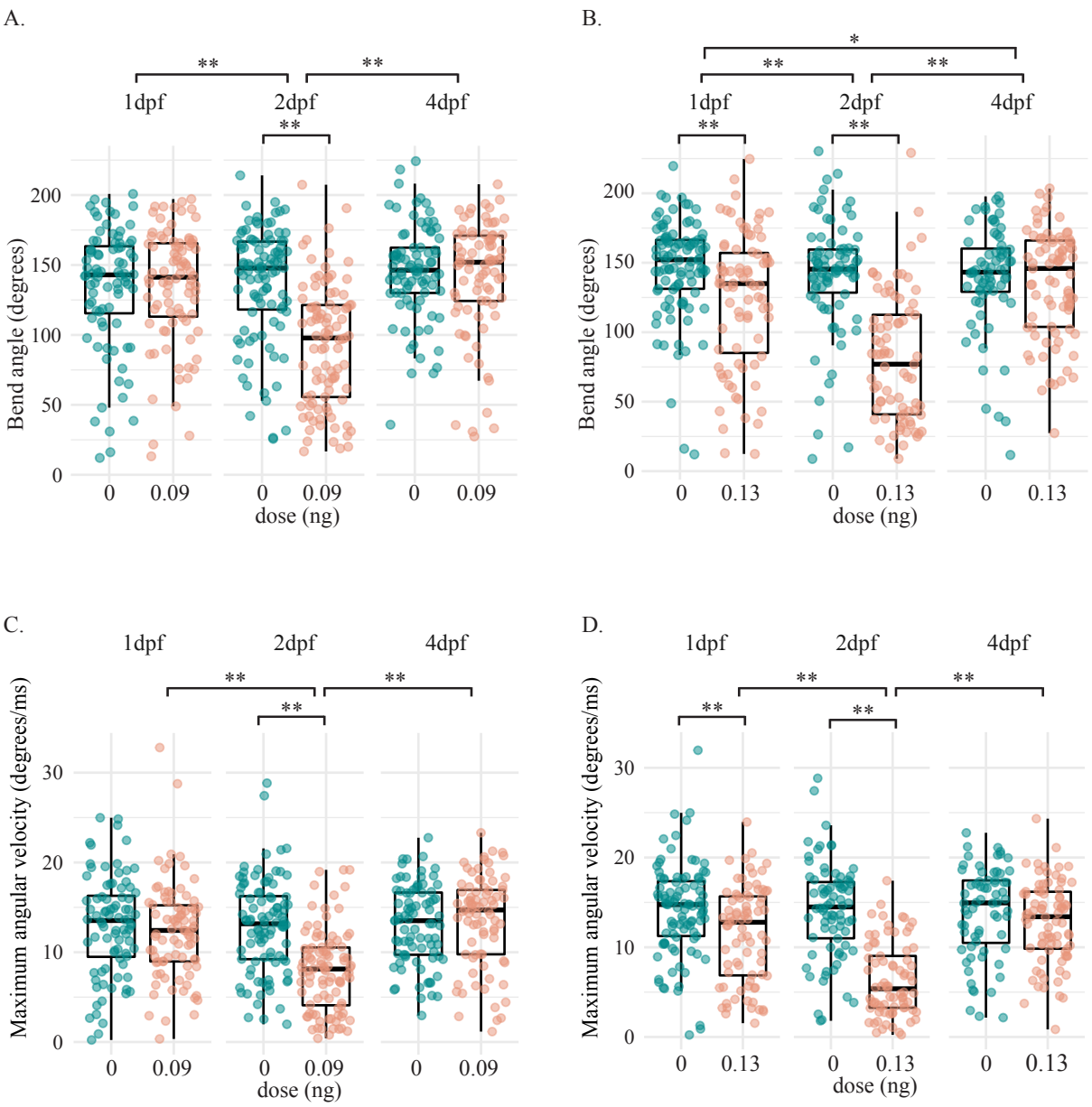


Figure S4: Startle kinematics comparisons among zebrafish exposed at 1, 2 and 4 dpf

(A) Bend angles during long latency c-bend (LLC) startle responses in fish exposed during different exposure days (1, 2 and 4 dpf) to the nominal dose of 0.09 ng of domoic acid (DomA) and **(B)** to a dose that ranged from 0.126- 0.144 (labelled as 0.13ng of DomA). **(C)** Maximal angular velocity during LLC startle responses in fish exposed during different exposure days to 0.09 ng of DomA and to **(D)** 0.13ng of DomA. Each point represents the median kinematic response of an individual larva to multiple identical stimuli. Boxplots show the group medians, upper 75% quantiles, and lower 25% quantiles. For the analysis of bend angles (S4A and S4B),

statistical significance was determined using Aligned Ranked Transformed analysis of variance (ANOVA) tests followed by difference-of-difference contrasts and Mann-Whitney U tests with the Holm-Bonferroni method to correct for multiple comparisons. For the analysis of maximal angular velocities (S4C and S4D), statistical significance was determined using two-way analysis of variance followed by Tukey's post-hoc tests (* $p < 0.05$, **=$p < 0.01$).

Figure supplement: Supplemental video 1 is a sample startle response.

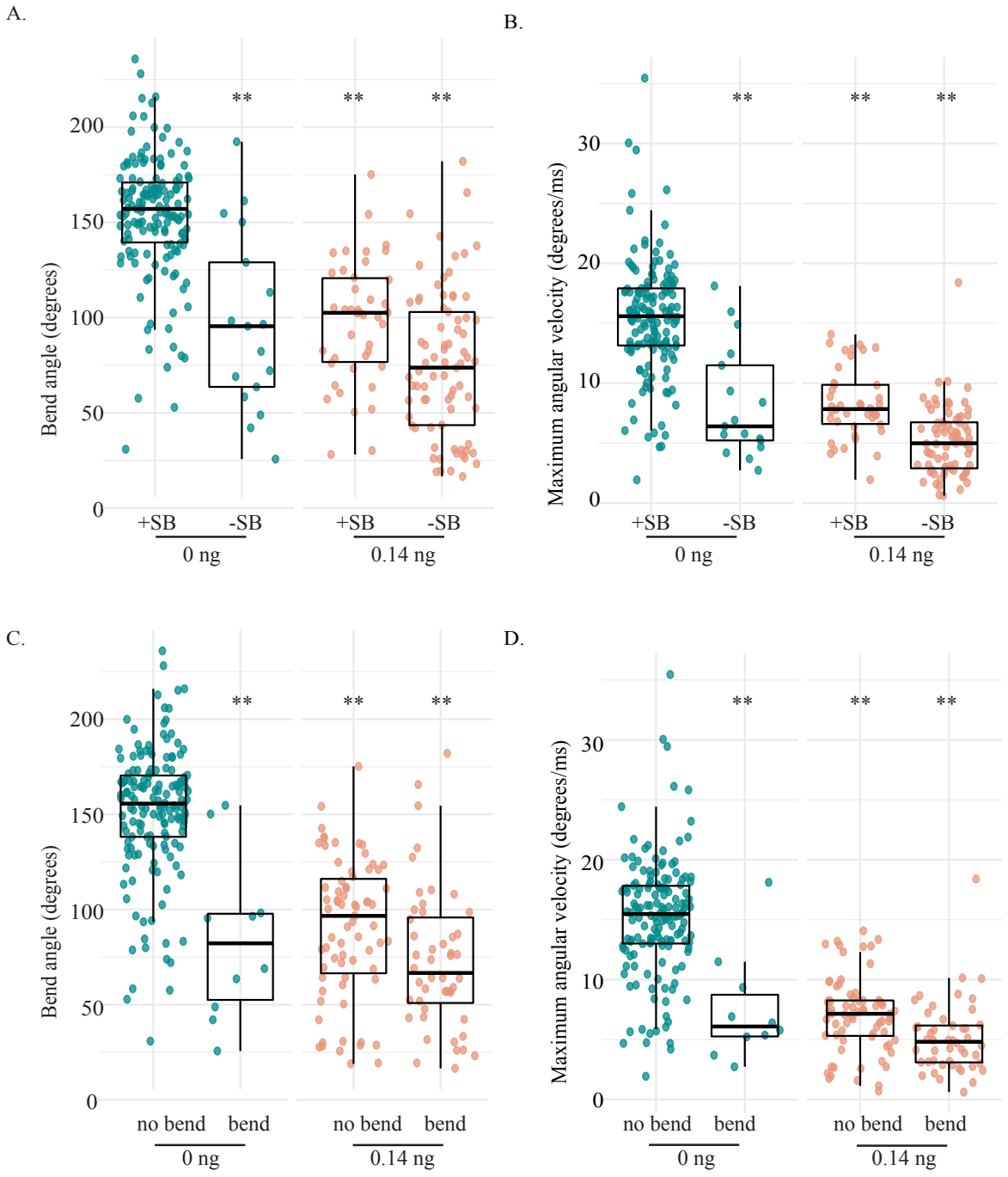


Figure S5: Startle kinematics comparisons with zebrafish exposed at 2 dpf with differing morphological attributes

Bend angles **(A)** and maximal angular velocities **(B)** during long latency c-bends (LLC) startles were characterized in control and DomA-exposed fish which either had an inflated swim bladder (+SB) or did not (-SB). Bend angles **(C)** and maximal angular velocities **(D)** during long latency c-bends (LLC) startles were also characterized in control and DomA-exposed fish which had a

straight body axis (no bend) or had a bent body axis (bend). Fish were exposed at 2 dpf to either vehicle saline (0 ng) or DomA (0.14ng). Statistical significance was determined using nonparametric multiple comparison procedures with Dunnett-type contrasts. All treatment groups were compared to controls with "control-like" phenotypes defined as: controls with inflated swim bladders (+SB) (for A and B) or controls with straight body axes (no bend) (for C and D) (**$p < 0.001$). Table S18 and S19 contain the results for the statistical analysis.

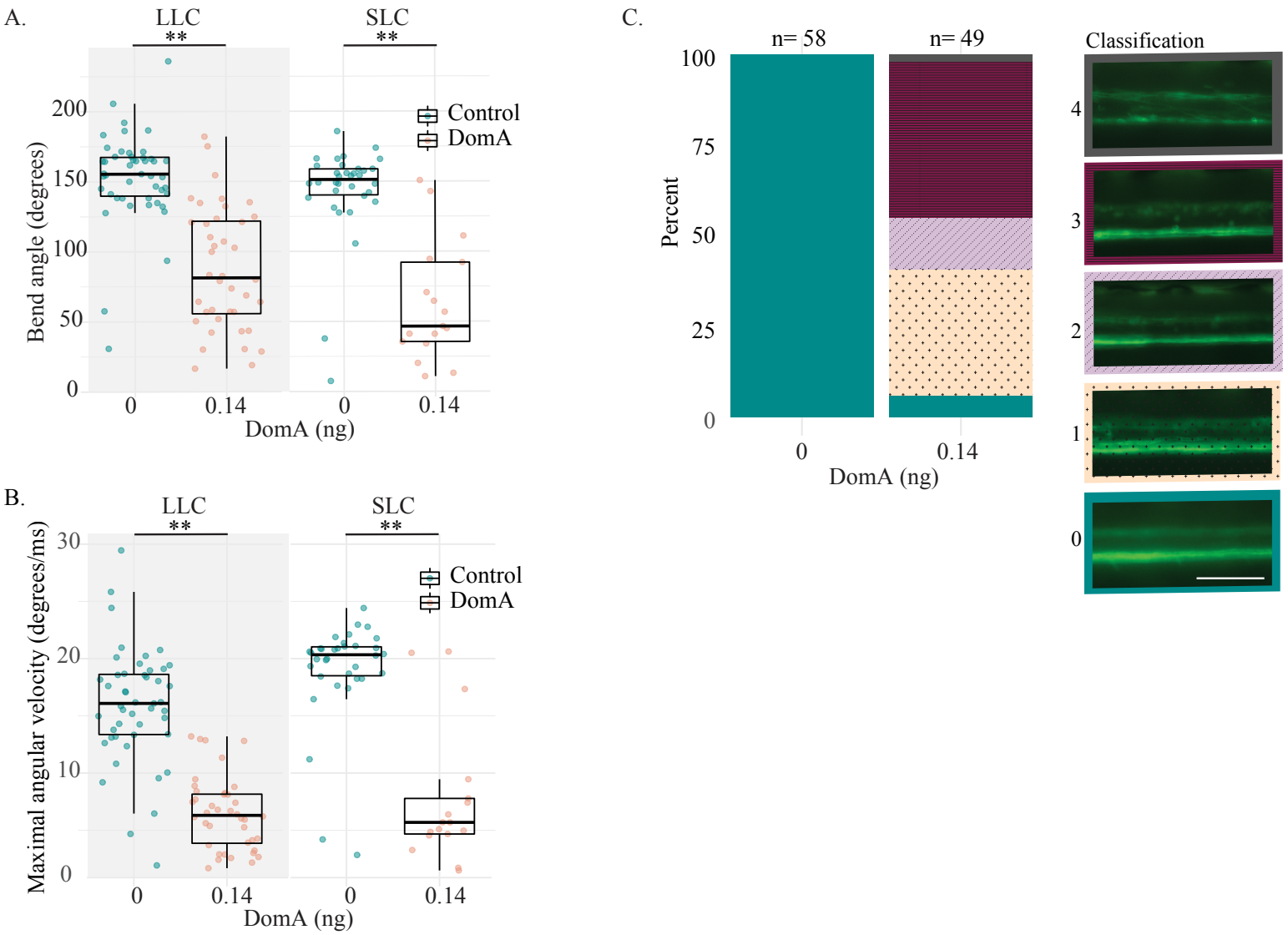


Figure S6: Startle kinematics and myelin sheath imaging in fish used for RNASeq

Tanks of 3 adult fish (2 females, 1 male) of *Tg(mbp:EGFP-CAAX)* background were bred and the embryos exposed to domoic acid (DomA) (0.14 ng) or vehicle at 2 dpf. Myelin sheath labeling was assessed at 5 dpf and startle response was assessed at 7 days post fertilization (dpf) prior to RNA sequencing.

Fish were exposed to 0.14 ng of domoic acid (DomA) at 2 day post fertilization (dpf). Long latency (LLC) and short latency (SLC) c-bends startle responses were characterized by bend angle (**A)** and maximal angular velocity (**B**). Each point represents the median of up to 7 responses for an individual fish. Boxplots show the group medians, upper 75% quantiles, and lower 25% quantiles. Significance was determined using Behrens-Fisher t-tests. Asterisks represent statistical significance of kinematics of DomA-exposed fish compared with controls (** $p < .001$). **(C)** Distribution of myelin phenotypes in control (0 ng) and DomA-treated fish exposed at 2 dpf and imaged at 5 dpf using widefield epifluorescence microscopy. Images were blindly classified into 6 categories based on severity of the observed myelin phenotype. The scoring was as described in detail in Figure S2. Briefly, the classification was: (0) normal phenotype, (1) myelin sheaths present but disorganized, (2) myelin with noticeable deficits, (3) myelin gaps in ventral spinal cord, (4) myelin sheaths lacking in ventral spinal cord, (5) visible sloughed myelin. Numbers above denote the total number of fish per treatment group. Boxplots show the group medians, upper 75% quantiles, and lower 25% quantiles.

(** = $p < 0.001$). ; Scale bar = 100 μm