Supplementary Information:

Locomotion dependent neuron-glia interactions control neurogenesis and regeneration in the adult zebrafish spinal cord

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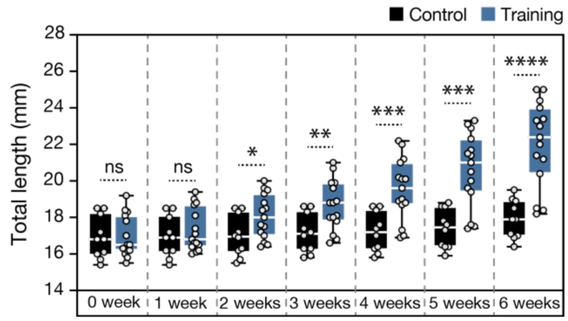
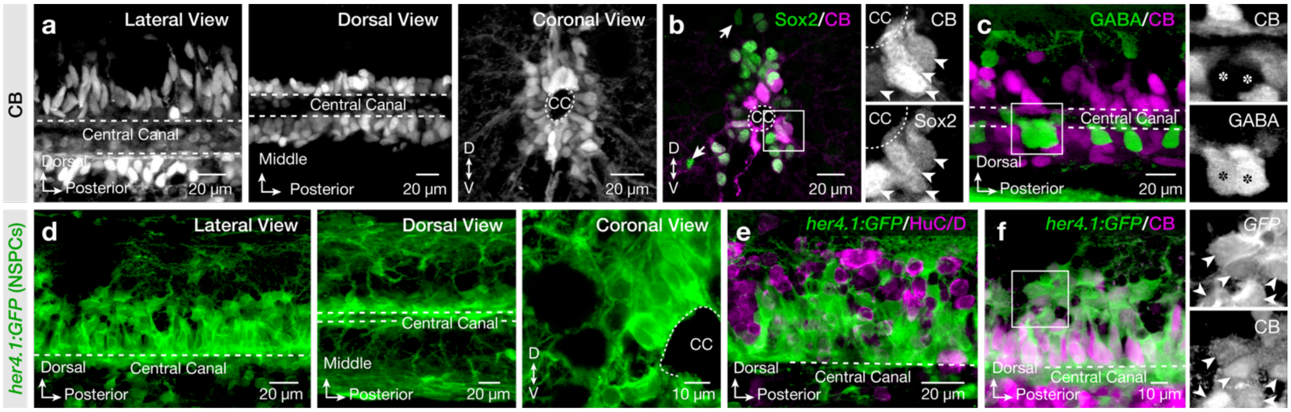
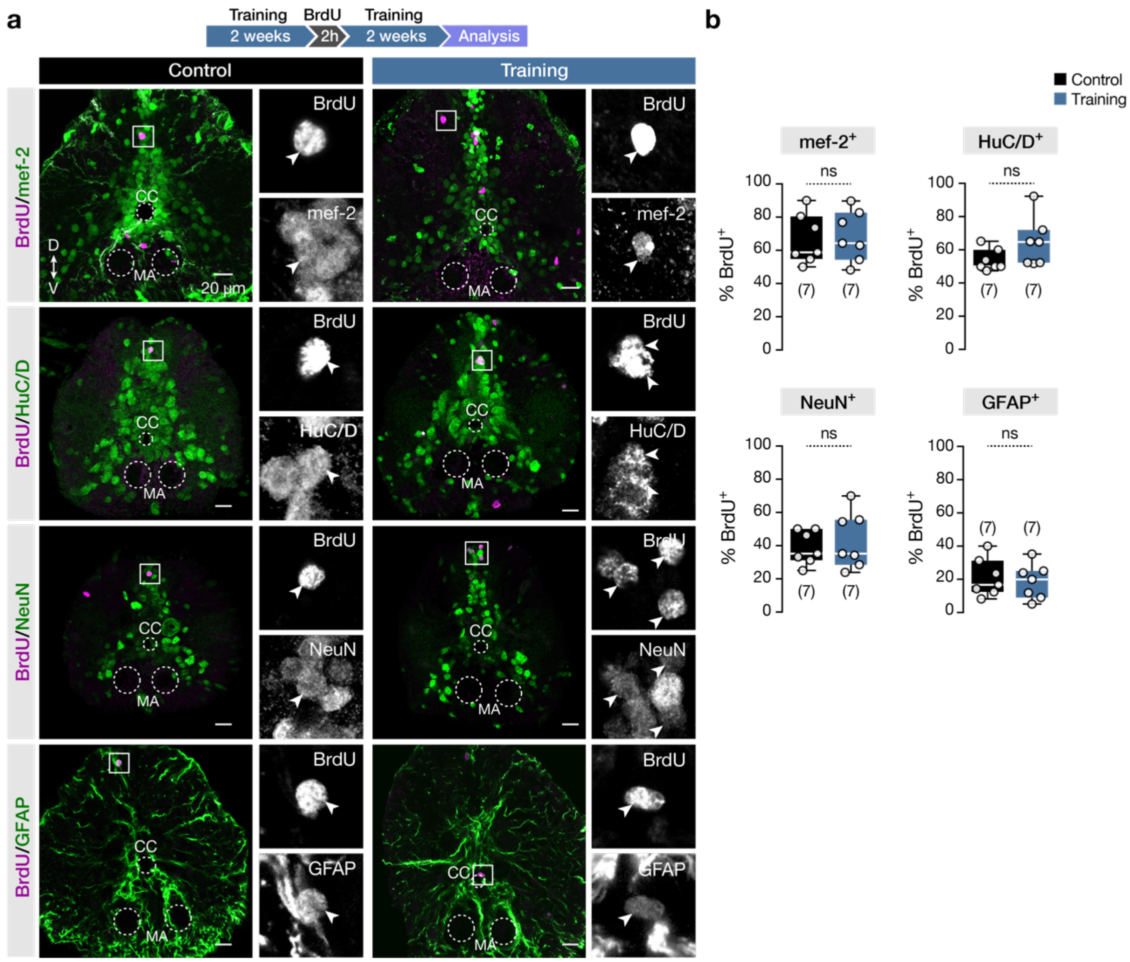
Supplementary figures:



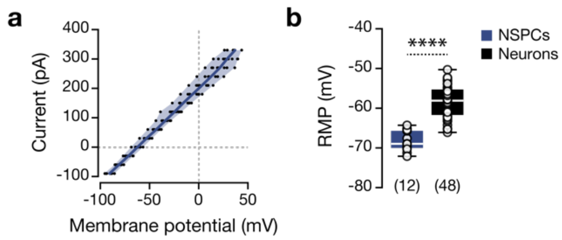
Supplementary Fig. 1. Physical activity increases animal growth. Quantification of the animal total length (TL) between the untrained (control; n=10) and trained (n=15) adult zebrafish. Data are presented as box plots showing the median with 25/75 percentile (box and line) and minimum–maximum (whiskers). *P<0.05; **P<0.01; ***P<0.001; ****P<0.0001; ns, not significant. For detailed statistics, see supplementary Table 1.



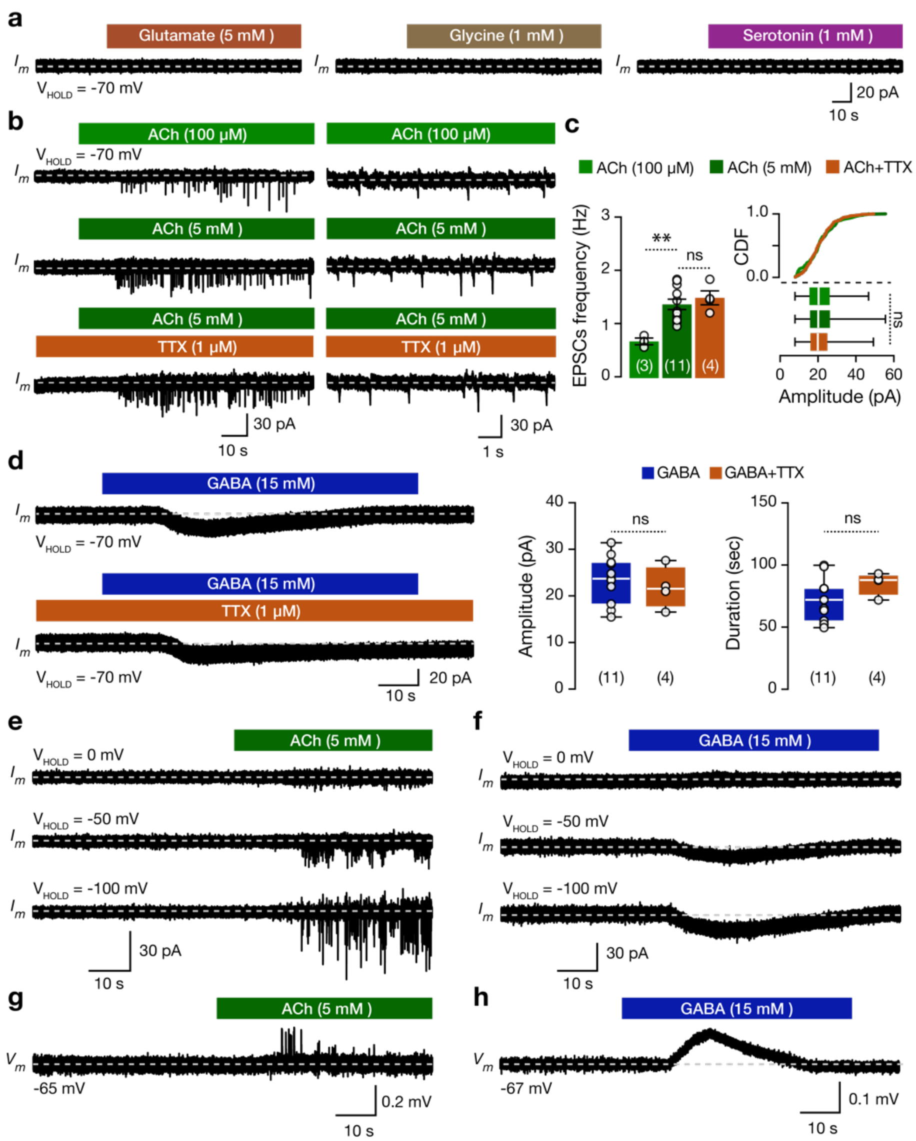
Supplementary Fig. 2. Identification of spinal cord neural stem/progenitor cells (NSPCs). a Distribution pattern of the calbindin^{+} (CB^{+}) immunolabeled cells in the central canal (cc) area. **b** High colocalization of CB (magenta) with the stem cell marker Sox2 (green). Arrows indicate the Sox2^{+}CB^{-} cells. Arrowheads in the inset indicate the double-labeled cells. **c** The cerebrospinal fluid contacting neurons (GABA^{+}, green) do not express CB (magenta). Asterisks indicate the GABA^{+}CB^{-} neurons. **d** Expression pattern of *GFP* (green) driven by the radial glial promoter *her4.1* in the adult zebrafish spinal cord defining the spinal NSPCs. **e** GFP^{+} NSPCs (green) do not express the neuronal marker HuC/D (magenta). **f** All *her4.1*$^{+}$ cells (NSPCs, green) are CB^{+} (magenta). Arrowheads in the inset indicate the double-labeled cells. CB, calbindin D-28K; CC, central canal; D, dorsal; GABA, γ-aminobutyric acid; GFP, green fluorescent protein; her4.1, hairy-related 4, tandem duplicate 1; HuC/D, elav3+4; NSPC, neural stem/progenitor cell; Sox2, sex-determining region Y-box 2; V, ventral.



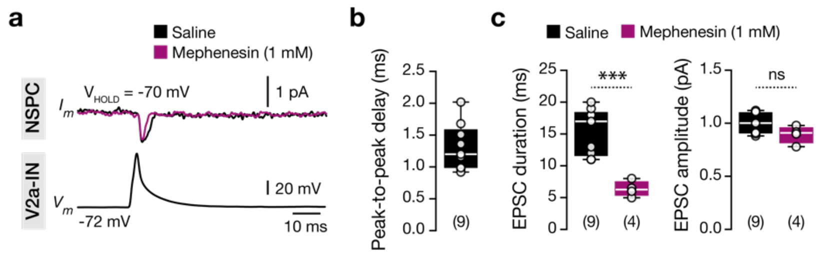
Supplementary Fig. 3. Differentiation profile of BrdU⁺ cells. a Coronal sections of the adult zebrafish spinal cord used for immunodetection of BrdU⁺ cells (magenta), expressing neuronal (mef-2, HuC/D, NeuN; green) or GFAP (green) markers in control and trained animals. Arrowheads indicate double-labeled cells. **b** Quantification of the proportion of the BrdU⁺ cells that are also mef-2⁺, HuC/D⁺, NeuN⁺, or GFAP⁺ in control and trained animals. A majority of BrdU⁺ cells express neuronal markers indication neuronal differentiation. BrdU, 5-Bromo-2'-Deoxyuridine; CC, central canal; D, dorsal; MA, GFAP, Glial fibrillary acidic protein; Mauthner axon; mef-2, myocyte enhancer factor-2; NeuN, neuronal nuclei; V, ventral. Data are presented as box plots showing the median with 25/75 percentile (box and line) and minimum–maximum (whiskers). ns, not significant. For detailed statistics, see supplementary Table 1.



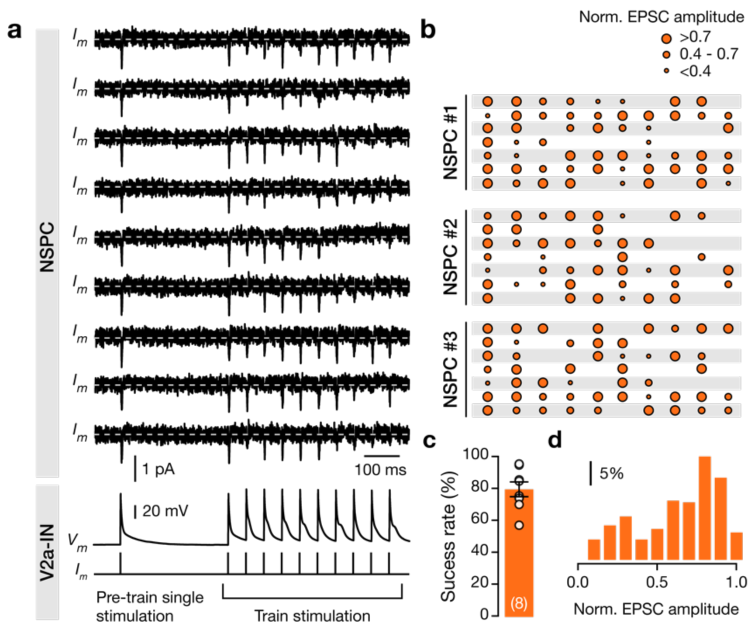
Supplementary Fig. 4. Cellular properties of spinal NSPCs. a Current-Voltage (I-V) relationships for the NSPCs (*her4.1:GFP*⁺) obtained from current-clamp recordings. **b** Resting membrane potential differences between the NSPCs (*her4.1:GFP*⁺) and spinal cord neurons (V2a-INs and motoneurons; *n*: number of recorded cells; *t*-test: t = 8.226, df = 58, *P*=2.563E-11). NSPC, neural stem/progenitor cell; RMP, resting membrane potential. Data are presented as box plots showing the median with 25/75 percentile (box and line) and minimum–maximum (whiskers). *****P*<0.0001. For detailed statistics, see supplementary Table 1.



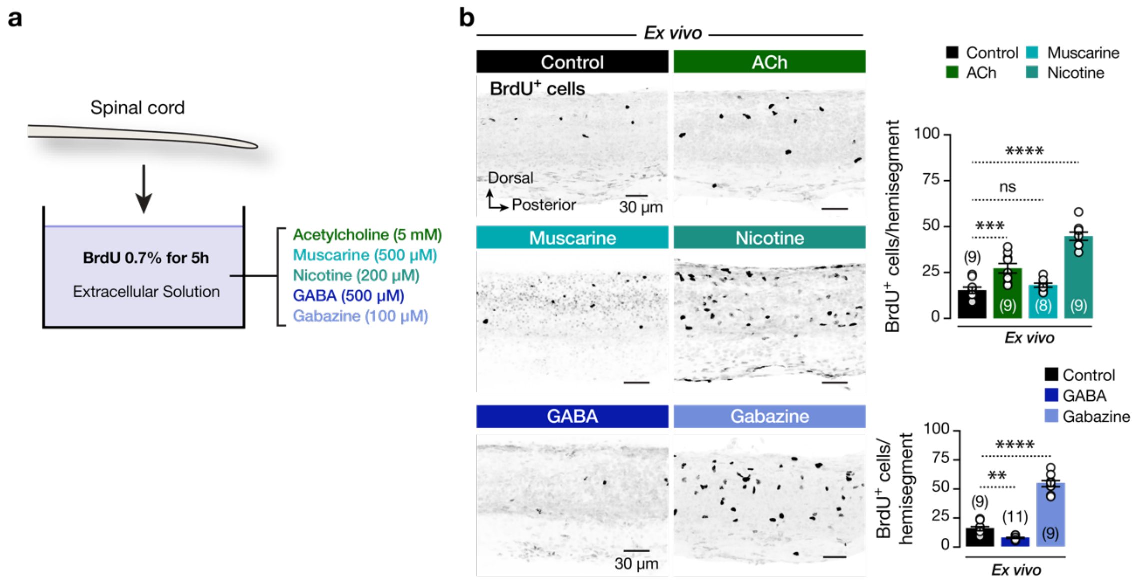
Supplementary Fig. 5. Cholinergic and GABAergic responses by NSPCs in the adult spinal cord. a Voltage-clamp recordings followed by bath application of glutamate (0 out of 8), glycine (0 out of 6), and serotonin (0 out of 7) do not evoke any apparent response in NSPCs. **b** Representative traces show the induced currents in NSPCs after bath application of ACh (100μM or 5mM) and in the presence of TTX (1 μM). (**c**) ACh treatment induced a dose-dependent increase in EPSC frequency, but the amplitudes of the EPSCs were independent of the ACh concentration or the presence of TTX (*n*: number of recorded NSPCs). **d** Sample traces show the induced tonic activity currents in NSPCs after bath application of GABA (5mM) in presence of TTX (1 μM). Quantification of the response amplitude and duration in control and TTX (*n*: number of recorded NSPCs). **e-f** Sample traces showing the differences between the ACh and GABA induced responses in the spinal cord NSPCs at different holding potentials. **g-h** Current-clamp NSPCs recordings show that ACh application generated many EPSPs, whereas GABA induced a tonic membrane depolarization. ACh, acetylcholine; CDF, cumulative distribution frequencies; GABA, γ-aminobutyric acid; TTX, tetrodotoxin. The dashed gray line represents the baseline. Data are presented as means ± s.e.m. and as box plots showing the median with 25/75 percentile (box and line) and minimum-maximum (whiskers). $^{**}P<0.01$; ns, not significant. For detailed statistics, see supplementary Table 1.



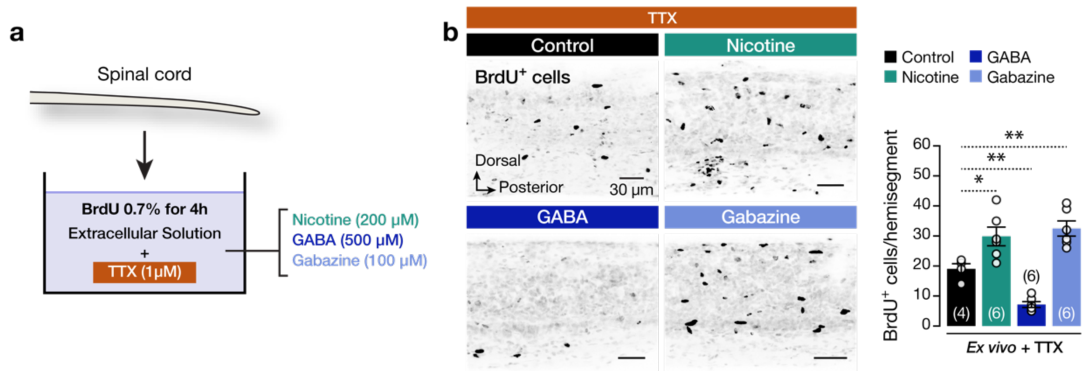
Supplementary Fig. 6. Analysis of the inter-segmental connectivity between the V2a-INs and the NSPCs. a Example traces (lowpass filtered) showing the changes in the evoked EPSCs in a postsynaptic NSPC in control (saline, black trace) and after application of the potential polysynaptic blocker mephenesin (magenta). **b** Analysis of the synaptic delay between the V2a-INs and the NSPCs postsynaptic responses suggesting that are likely monosynaptic (*n*: number of recorded NSPC/V2a-IN pairs). **c** Quantification of EPSCs duration and amplitude before and after the application of mephenesin suggesting a mixture of monosynaptic (direct) and polysynaptic (indirect) connections (*n*: number of recorded NSPC/V2a-IN pairs). NSPC, neural stem/progenitor cell. Data are presented as box plots showing the median with 25/75 percentile (box and line) and minimum-maximum (whiskers). ****P*<0.001; ns, not significant. For detailed statistics, see supplementary Table 1.



Supplementary Fig. 7. Synaptic connection reliability between V2a-INs and the NSPCs. a Example traces of individual sweeps (*n*=9) showing the failures and changes in the evoked EPSCs in postsynaptic stem/progenitor cells during the stimulation train. **b** Analysis from three V2a-IN / NSPC pairs showing the failures and the normalized EPSC amplitude changes during the train of action potentials evoked in V2a-INs. **c-d** Success rate analysis (%) and frequency distribution of the evoked EPSC amplitudes during the train of action potential evoked in V2a-INs (*n*: number of recorded NSPC/V2a-IN pairs). In all cases, the EPSC amplitudes were normalized to the EPSC amplitude of the pre-train single stimulation. The dashed gray line represents the baseline. NSPC, neural stem/progenitor cell. Data are presented as means ± s.e.m. For detailed statistics, see supplementary Table 1.



Supplementary Fig. 8. *Ex vivo* cholinergic and GABAergic modulation of proliferation in the spinal cord. a Experimental setup for the *ex vivo* investigation of proliferation in intact isolated spinal cords. **b** Whole-mount confocal microphotographs illustrating BrdU-incorporation per hemisegment following pharmacological treatments and quantifications. ACh, muscarine, and nicotine increased the number of BrdU$^+$ cells. Administration of GABA or gabazine indicated a role for $GABA_A$ receptors in maintaining NSPC quiescence. ACh, acetylcholine; BrdU, 5-Bromo-2'-Deoxyuridine; GABA, γ-aminobutyric acid. Data are presented as means ± s.e.m. ***P*<0.01; ****P*<0.001; *****P*<0.0001; ns, not significant. For detailed statistics, see supplementary Table 1.



Supplementary Fig. 9. ***Ex vivo*** **cholinergic and GABAergic modulation of spinal cord proliferation in spike eliminating environment. a** Experimental setup for the *ex vivo* investigation of proliferation in intact isolated spinal cords in a presence of tetrodotoxin. **b** Whole-mount confocal microphotographs illustrating the detected BrdU$^+$ per hemisegment following pharmacological treatments and followed by quantifications. BrdU, 5-Bromo-2'-Deoxyuridine; GABA, γ-aminobutyric acid; TTX, tetrodotoxin. Data are presented as means ± s.e.m. **P*<0.05; ***P*<0.01. For detailed statistics, see supplementary Table 1.

Supplementary Table 1. Detailed statistics

Figure	Statistics	Result	Post-hoc Test	comparison	Significance	*P-value*
1c	One-way ANOVA	$F_{(2, 24)} = 60.23$, $P < 0.0001$	Dunnett's test	Control Training	****	P_{adj} <0.0001
				Control Rest	ns	P_{adj} = 0.206
	Descriptive	Control (*n* = 10 zebrafish): 14.5 ± 1.376 Training (*n* = 9 zebrafish): 42.44 ± 3.532 Rest (*n* = 8 zebrafish): 9.25 ± 0.959				
1f	Unpaired *t*-test	t = 3.946, df = 10 (Two-tailed)		Control Training	**	*P* = 0.0027
	Descriptive	Control (*n* = 5 zebrafish): 1.66 ± 0.235 Training (*n* = 7 zebrafish): 4.09 ± 0.486				
1g mef-2	Unpaired *t*-test	t = 6.495, df = 12 (Two-tailed)		Control Training	****	*P* < 0.0001
	Descriptive	Control (*n* = 7 zebrafish): 0.952 ± 0.086 Training (*n* = 7 zebrafish): 2.69 ± 0.253				
1g HuC/D	Unpaired *t*-test	t = 5.536, df = 12 (Two-tailed)		Control Training	***	*P* = 0.0001
	Descriptive	Control (*n* = 7 zebrafish): 0.857 ± 0.111 Training (*n* = 7 zebrafish): 2.19 ± 0.213				
1g NeuN	Unpaired *t*-test	t = 4.475, df = 12 (Two-tailed)		Control Training	***	*P* = 0.0008
	Descriptive	Control (*n* = 7 zebrafish): 0.595 ± 0.071 Training (*n* = 7 zebrafish): 1.619 ± 0.217				
2d	Linear regression	$R^2 = 0.2124$ Sy.x = 1.296 Equation: Y= -0.6834*X + 7.865				
2e	Linear regression	$R^2 = 0.9098$ Sy.x = 0.4203 Equation: Y=0.3552*X – 1.09				
3a Frequency	One-way ANOVA	$F_{(2, 22)} = 17.15$, $P < 0.0001$	Tukey's test	ACh Muscarine	****	P_{adj} < 0.0001
				ACh Nicotine	ns	P_{adj} = 0.9978
				Muscarine Nicotine	***	P_{adj} = 0.0001
	Descriptive	ACh (*n* = 11 cells): 1.362 ± 0.098 Muscarine (*n* = 6 cells): 0.694 ± 0.045 Nicotine (*n* = 8 cells): 1.355 ± 0.054				
3a Amplitude	One-way ANOVA	$F_{(2, 573)} = 66.56$, $P < 0.0001$	Tukey's test	ACh Muscarine	****	P_{adj} < 0.0001
				ACh Nicotine	ns	P_{adj} = 0.4183
				Muscarine Nicotine	****	P_{adj} < 0.0001
	Descriptive	ACh (*n* = 294 EPSCs): 21.48 ± 0.46 Muscarine (*n* = 88 EPSCs): 11.85 ± 0.295 Nicotine (*n* = 194 EPSCs): 20.67 ± 0.494				
3b Amplitude	One-way ANOVA	$F_{(2, 18)} = 46.38$, $P < 0.0001$	Tukey's test	GABA Muscimol	ns	P_{adj} = 0.616
				GABA GABA + Gabazine	****	P_{adj} < 0.0001
				Muscimol GABA + Gabazine	****	P_{adj} < 0.0001
	Descriptive	GABA (*n* = 11 cells): 23.55 ± 1.524 Muscimol (*n* = 6 cells): 25.75 ± 2.0 GABA + Gabazine (*n* = 4 cells): 0.0 ± 0.0				
3b Duration	One-way ANOVA	$F_{(2, 18)} = 44.16$, $P < 0.0001$	Tukey's test	GABA Muscimol	ns	P_{adj} = 0.9451

				GABA GABA + Gabazine	****	$P_{adj} < 0.0001$
				Muscimol GABA + Gabazine	****	$P_{adj} < 0.0001$
	Descriptive	GABA (*n* = 11 cells): 72.73 ± 5.233 Muscimol (*n* = 6 cells): 75.02 ± 4.319 GABA + Gabazine (*n* = 4 cells): 0.0 ± 0.0				
3c μ EPSCs number	Paired *t*-test	t = 2.584, df = 4 (Two-tailed)		Control Mephenesin	ns	$P = 0.0610$
	Descriptive	Control (*n* = 5 cells): 0.804 ± 0.057 Mephenesin (*n* = 5 cells): 0.678 ± 0.042				
3c μ EPSCs amplitude	Paired *t*-test	t = 0.191, df = 4 (Two-tailed)		Control Mephenesin	ns	$P = 0.8575$
	Descriptive	Control (*n* = 5 cells): 8.608 ± 0.985 Mephenesin (*n* = 5 cells): 8.553 ± 0.964				
4b	Descriptive	(*n* = 8 zebrafish): 41.81 ± 3.62				
4d	Descriptive	(*n* = 8 zebrafish) V2a-INs: 25 out of 25 neurons (100%) Non V2a-INs: 0 out of 25 neurons (0%)				
4e Number of neurons	Descriptive	(*n* = 8 zebrafish) Number of neurons/hemisegment: 3.125 ± 0.226				
4e Soma size	Descriptive	(*n* = 25 neurons from 8 zebrafish) 78.99 ± 3.189				
4f Connectivity	Descriptive	Intra-segmental: 0 out of 15 (0.0%) Inter-segmental: 11 out of 50 (22%)				
4i responses	Descriptive	Control (*n* = 9 cells) Responding: 3 (37.5%) Not Responding: 5 (62.5%) Training (*n* = 12 cells) Responding: 7 (58.33%) Not Responding: 5 (41.67%)				
4i EPSCs	Unpaired *t*-test	t = 9.524, df = 210 (Two-tailed)		Control Training	****	$P < 0.0001$
	Descriptive	Control (*n* = 87): 0.942 ± 0.082 Training (*n* = 125): 2.376 ± 0.111				
5a	One-way ANOVA	$F_{(3, 215)} = 59.88$, $P < 0.0001$	Tukey's test	Saline Nicotine	****	$P_{adj} < 0.0001$
				Saline GABA	*	$P_{adj} = 0.013$
				Saline Gabazine	***	$P_{adj} = 0.0004$
				Nicotine GABA	****	$P_{adj} < 0.0001$
				Nicotine Gabazine	ns	$P_{adj} = 0.265$
				GABA Gabazine	****	$P_{adj} < 0.0001$
	Descriptive	Saline (*n* = 4 zebrafish): 1.917 ± 0.515 Nicotine (*n* = 5 zebrafish): 5.267 ± 0.286 GABA (*n* = 6 zebrafish): 0.383 ± 0.183 Gabazine (*n* = 4 zebrafish): 4.417 ± 0.25				
5b Cholinergic	One-way ANOVA	$F_{(3, 32)} = 128.4$, $P < 0.0001$	Dunnett's test	Saline ACh	****	$P_{adj} < 0.0001$
				Saline Muscarine	**	$P_{adj} = 0.0023$
				Saline Nicotine	****	$P_{adj} < 0.0001$
	Descriptive	Saline (*n* = 13 zebrafish): 17.85 ± 1.372 ACh (*n* = 9 zebrafish): 34.44 ± 2.873 Muscarine (*n* = 8 zebrafish): 29 ± 1.464				

		Nicotine (*n* = 6 zebrafish): 81.67 ± 3.739				
5b GABAergic	One-way ANOVA	$F_{(2, 21)}$ = 270.2, P < 0.0001	Dunnett's test	Saline GABA	***	P_{adj} = 0.0002
				Saline Gabazine	****	P_{adj} < 0.0001
	Descriptive	Saline (*n* = 13 zebrafish): 17.85 ± 1.372 GABA (*n* = 6 zebrafish): 3.66 ± 1.909 Gabazine (*n* = 5 zebrafish): 81.6 ± 4.155				
5c	One-way ANOVA	$F_{(4, 31)}$ = 141.3, P < 0.0001	Tukey's test	Saline Nicotine+Gabazine	****	P_{adj} < 0.0001
				Saline Nicotine	****	P_{adj} < 0.0001
				Saline Gabazine	****	P_{adj} < 0.0001
				Saline Nicotine+GABA	ns	P_{adj} = 0.4206
				Nicotine Gabazine	ns	P_{adj} > 0.9999
				Nicotine Nicotine+Gabazine	ns	P_{adj} = 0.9814
				Nicotine Nicotine+GABA	****	P_{adj} < 0.0001
				Gabazine Nicotine+Gabazine	ns	P_{adj} = 0.9828
				Gabazine Nicotine+GABA	****	P_{adj} < 0.0001
				Nicotine+Gabazine Nicotine+GABA	****	P_{adj} < 0.0001
	Descriptive	Saline (*n* = 13 zebrafish): 17.85 ± 1.372 Nicotine+gabazine (*n* = 6 zebrafish): 84.17 ± 3.28 Nicotine (*n* = 6 zebrafish): 81.67 ± 3.739 Gabazine (*n* = 5 zebrafish): 81.6 ± 4.155 Nicotine+GABA (*n* = 6 zebrafish): 24.67 ± 4.302				
5d Frequency	Unpaired *t*-test	t = 0.418, df = 16 (Two-tailed)		Control Training	ns	P = 0.6809
	Descriptive	Control (*n* = 11 cells): 1.362 ± 0.098 Training (*n* = 7 cells): 1.429 ± 0.1253				
5d Amplitude	Unpaired *t*-test	t = 0.379, df = 543 (Two-tailed)		Control Training	ns	P = 0.3791
	Descriptive	Control (*n* = 325 EPSCs): 20.97 ± 0.436 Training (*n* = 220 EPSCs): 20.39 ± 0.48				
5e Amplitude	Unpaired *t*-test	t = 4.295, df = 15 (Two-tailed)		Control Training	***	P = 0.0006
	Descriptive	Control (*n* = 11 cells): 23.55 ± 1.524 Training (*n* = 6 cells): 13.1 ± 1.723				
5e Duration	Unpaired *t*-test	t = 1.351, df = 15 (Two-tailed)		Control Training	ns	P = 0.1967
	Descriptive	Control (*n* = 11 cells): 72.73 ± 5.233 Training (*n* = 6 cells): 58.75 ± 10.36				
6d	One-way ANOVA	$F_{(2, 16)}$ = 7.954, P = 0.004	Dunnett's test	Saline Nicotine	**	P_{adj} = 0.0026
				Saline Gabazine	*	P_{adj} = 0.0336
	Descriptive	Saline (*n* = 7 zebrafish): 56.86 ± 2.521 Nicotine (*n* = 6 zebrafish): 74.0 ± 4.091 Gabazine (*n* = 6 zebrafish): 68.5 ± 2.849				
6e	One-way ANOVA	$F_{(2, 16)}$ = 5.741, P = 0.0132	Dunnett's test	Saline Nicotine	*	P_{adj} = 0.0127
				Saline Gabazine	*	P_{adj} = 0.0335
	Descriptive	Saline (*n* = 7 zebrafish): 28.86 ± 2.314				

		Nicotine (*n* = 6 zebrafish): 38.67 ± 2.642 Gabazine (*n* = 6 zebrafish): 37.17 ± 1.641				
6f	One-way ANOVA	$F_{(2, 16)}$ = 9.548, *P* = 0.0019	Dunnett's test	Saline Nicotine	**	P_{adj} = 0.0011
				Saline Gabazine	*	P_{adj} = 0.0326
	Descriptive	Saline (*n* = 7 zebrafish): 4.829 ± 0.541 Nicotine (*n* = 6 zebrafish): 9.5 ± 0.928 Gabazine (*n* = 6 zebrafish): 7.7 ± 0.869				
Supplementary Figures						
Suppl 1 0 week	Unpaired *t*-test	t = 0.043, df = 23 (Two-tailed)		Control Training	ns	*P* = 0.9660
	Descriptive	Control (*n* = 10 zebrafish): 16.92 ± 0.366 Training (*n* = 15 zebrafish): 16.94 ± 0.29				
Suppl 1 1 week	Unpaired *t*-test	t = 0.775, df = 23 (Two-tailed)		Control Training	ns	*P* = 0.4459
	Descriptive	Control (*n* = 10 zebrafish): 16.95 ± 0.35 Training (*n* = 15 zebrafish): 17.31 ± 0.3				
Suppl 1 2 weeks	Unpaired *t*-test	t = 2.193, df = 23 (Two-tailed)		Control Training	*	*P* = 0.0387
	Descriptive	Control (*n* = 10 zebrafish): 17.01 ± 0.354 Training (*n* = 15 zebrafish): 18.06 ± 0.311				
Suppl 1 3 weeks	Unpaired *t*-test	t = 2.955, df = 23 (Two-tailed)		Control Training	**	*P* = 0.0071
	Descriptive	Control (*n* = 10 zebrafish): 17.14 ± 0.34 Training (*n* = 15 zebrafish): 18.66 ± 0.352				
Suppl 1 4 weeks	Unpaired *t*-test	t = 3.863, df = 23 (Two-tailed)		Control Training	***	*P* = 0.0008
	Descriptive	Control (*n* = 10 zebrafish): 17.21± 0.34 Training (*n* = 15 zebrafish): 19.53 ± 0.4336				
Suppl 1 5 weeks	Unpaired *t*-test	t = 4.65, df = 23 (Two-tailed)		Control Training	***	*P* = 0.0001
	Descriptive	Control (*n* = 10 zebrafish): 17.39 ± 0.335 Training (*n* = 15 zebrafish): 20.61 ± 0.517				
Suppl 1 6 weeks	Unpaired *t*-test	t = 5.244, df = 23 (Two-tailed)		Control Training	****	*P* < 0.0001
	Descriptive	Control (*n* = 10 zebrafish): 17.91 ± 0.33 Training (*n* = 15 zebrafish): 22.04 ± 0.6				
Suppl 3b Mef-2	Unpaired *t*-test	t = 0.243, df = 12 (Two-tailed)		Control Training	ns	*P* = 0.811
	Descriptive	Control (*n* = 7 zebrafish): 66.53 ± 5.64 Training (*n* = 7 zebrafish): 68.49 ± 5.719				
Suppl 3b HuC/D	Unpaired *t*-test	t = 1.751, df = 12 (Two-tailed)		Control Training	ns	*P* = 0.105
	Descriptive	Control (*n* = 7 zebrafish): 53.77 ± 2.453 Training (*n* = 7 zebrafish): 64.36 ± 5.525				
Suppl 3b NeuN	Unpaired *t*-test	t = 0.6, df = 12 (Two-tailed)		Control Training	ns	*P* = 0.559
	Descriptive	Control (*n* = 7 zebrafish): 38.73 ± 3.763 Training (*n* = 7 zebrafish): 43.19 ± 6.405				
Suppl 3b GFAP	Unpaired *t*-test	t = 0.391, df = 12 (Two-tailed)		Control Training	ns	*P* = 0.7
	Descriptive	Control (*n* = 7 zebrafish): 20.94 ± 4.282 Training (*n* = 7 zebrafish): 18.66 ± 3.977				
Suppl 4b	Unpaired *t*-test	t = 8.226, df = 58 (Two-tailed)		NSPCs Neurons	****	*P* < 0.0001
	Descriptive	NSPCs (*n* = 12): -68.27 ± 0.749 Neurons (*n* = 48): -58.31 ± 0.573				
Suppl 5c Frequency	One-way ANOVA	$F_{(2, 10)}$ = 9.036, *P* = 0.0057	Tukey's test	ACh (100μM) ACh (5mM)	**	P_{adj} = 0.0095

				ACh (100μM) ACh (5mM) + TTX	**	P_{adj} = 0.0078
				ACh (5mM) ACh (5mM) + TTX	ns	P_{adj} = 0.88
	Descriptive	ACh (100μM): 0.666 ± 0.064 ACh (5mM): 1.4 ± 0.133 ACh (5mM) + TTX: 1.486 ± 0.13				
Suppl 5c Amplitude	One-way ANOVA	$F_{(2, 639)}$ = 0.111			ns	*P* = 0.895
	Descriptive	ACh (100μM): 21.22 ± 0.789 ACh (5mM): 21.48 ± 0.46 ACh (5mM) + TTX: 21.17 ± 0.493				
Suppl 5d Amplitude	Unpaired *t*-test	t = 0.559, df = 13 (Two-tailed)		GABA GABA + TTX	ns	*P* = 0.5594
	Descriptive	GABA (*n* = 11): 23.55 ± 1.524 GABA + TTX (*n* = 4): 21.82 ± 2.27				
Suppl 5d Duration	Unpaired *t*-test	t = 1.351, df = 13 (Two-tailed)		GABA GABA + TTX	ns	*P* = 0.1999
	Descriptive	GABA (*n* = 11): 72.73 ± 5.233 GABA + TTX (*n* = 4): 85.23 ± 4.596				
Suppl 6b	Descriptive	(*n* = 9): 1.312 ± 0.122				
Suppl 6c Duration	Unpaired *t*-test	t = 4.753, df = 11 (Two-tailed)		Saline Mephenesin	***	*P* = 0.0006
	Descriptive	Saline (*n* = 9): 15.44 ± 1.215 Mephenesin (*n* = 4): 6.375 ± 0.625				
Suppl 6c Amplitude	Unpaired *t*-test	t = 1.784, df = 11 (Two-tailed)		Saline Mephenesin	ns	*P* = 0.1020
	Descriptive	Saline (*n* = 9): 1.002 ± 0.035 Mephenesin (*n* = 4): 0.895 ± 0.041				
Suppl 7c	Descriptive	Success rate (*n* = 8): 7.952 ± 0.461				
Suppl 8b Cholinergic	One-way ANOVA	$F_{(3, 31)}$ = 43.0, *P* < 0.0001	Dunnett's test	Control ACh	***	P_{adj} = 0.0006
				Control Muscarine	ns	P_{adj} = 0.6646
				Control Nicotine	****	P_{adj} < 0.0001
	Descriptive	Control (*n* = 9 zebrafish): 15.33 ± 1.74 ACh (*n* = 9 zebrafish): 27.33 ± 2.593 Muscarine (*n* = 8 zebrafish): 18.13 ± 1.141 Nicotine (*n* = 9 zebrafish): 44.78 ± 2.216				
Suppl 8b GABAergic	One-way ANOVA	$F_{(2, 26)}$ = 207.6, *P* < 0.0001	Dunnett's test	Control GABA	**	P_{adj} = 0.0056
				Control Gabazine	****	P_{adj} < 0.0001
	Descriptive	Control (*n* = 9 zebrafish): 15.33 ± 1.74 GABA (*n* = 11 zebrafish): 7.455 ± 0.511 Gabazine (*n* = 9 zebrafish): 54.11 ± 2.622				
Suppl 9b With TTX	One-way ANOVA	$F_{(3, 18)}$ = 26.12, *P* < 0.0001	Dunnett's test	Control Nicotine	*	P_{adj} = 0.0176
				Control GABA	**	P_{adj} = 0.0097
				Control Gabazine	**	P_{adj} = 0.0035
	Descriptive	Control (*n* = 4 zebrafish): 19.0 ± 1.78 Nicotine (*n* = 6 zebrafish): 29.83 ± 3.114 GABA (*n* = 6 zebrafish): 7.167 ± 0.98 Gabazine (*n* = 6 zebrafish): 32.5 ± 2.513				

Supplementary Table 2. Antibodies Used[1]

Antigen	Host	Source	Code	Dilution
Primary				
BrdU	Mouse	Becton Dickinson	347580; RRID: AB_10015219	1:80-1:100
ChAT	Goat	Millipore	AB144P; RRID: AB_2079751	1:200
Calbindin D-28K	Mouse	Swant	300; RRID: AB_2079751	1:400-1:1000
GABA	Rabbit	Sigma	A2052; RRID: AB_477652	1:2000
GFAP	Rabbit	Cell Signaling	12389; RRID: AB_2631098	1:200
GFP	Rabbit	Molecular Probes	A-11122; RRID: AB_221569	1:500
GFP	Chicken	Abcam	AB13970; RRID: AB_300798	1:600
HuC/D	Mouse	Molecular Probes	A-21271; RRID: AB_221448	1:100
HuC/D	Rabbit	GeneTex	GTX128365; RRID: N/A	1:500
Mef-2	Rabbit	Santa Cruz	SC313; RRID: AB_631920	1:50-1:80
NeuN	Rabbit	Cell Signaling	24307; RRID: AB_2651140	1:500
Sox2	Goat	R&D Systems	AF2018; RRID: AB_355110	1:500
Secondary				
Goat IgG-568	Donkey	ThermoFisher	A-11057; RRID: AB_2534104	1:500
Goat IgG-488	Donkey	ThermoFisher	A-11055; RRID: AB_2534102	1:500
Chicken IgY-FITC	Donkey	ThermoFisher	SA1-72000; RRID: AB_923386	1:800
Mouse IgG-647	Donkey	ThermoFisher	A-31571; RRID: AB_162542	1:500
Mouse IgG-568	Donkey	ThermoFisher	A-10037; RRID: AB_2534013	1:500
Mouse IgG-488	Donkey	ThermoFisher	A-21202; RRID: AB_141607	1:500
Rabbit IgG-488	Donkey	ThermoFisher	A-21206; RRID: AB_2535792	1:500
Rabbit IgG-647	Donkey	ThermoFisher	A-31573; RRID: AB_2536183	1:500
Rabbit IgG-568	Donkey	ThermoFisher	A-10042; RRID: AB_2534017	1:500

[1]BrdU, *5*-Bromo-*2*'-Deoxyuridine; ChAT, choline-acetyltransferase; mef-2, myocyte enhancer factor-2; GFAP, Glial fibrillary acidic protein; GABA, γ-aminobutyric acid; GFP, green fluorescent protein; PCNA, proliferating cell nuclear antigen; Sox2, sex determining region Y-box 2.