Supplemental Material

Table S1. RNA Quality Control Data

Sample	RIN	Raw reads #1	Raw reads #2	% bases Q>= 30	Uniquely mapped reads %	Multi-mapped reads %
TRAP control axons rep 1	7.8	29,430,720	29,430,720	94.48	77.47	19.04
TRAP control axons rep 2	8.0	27,285,154	27,285,154	95.24	78.08	18.39
TRAP control cortex rep 1	9.4	34,057,317	34,057,317	95.5	72.25	23.47
TRAP control cortex rep 2	9.8	38,634,382	38,634,382	94.96	70.66	25.54
TRAP trained axons rep 1	9.8	30,221,230	30,221,230	94.41	76.86	19.78
TRAP trained axons rep 2	8.7	27,951,448	27,951,448	94.32	76.68	19.66
TRAP trained cortex rep 1	9.9	37,791,175	37,791,175	94.79	69.93	25.90
TRAP trained cortex rep 2	9.7	34,481,070	34,481,070	94.91	72.18	23.83
Transc. control axons rep 1	6.4	35,934,968	35,934,968	93.03	87.30	10.10
Transc. control axons rep 2	7.2	36,774,857	36,774,857	95.05	87.42	9.98
Transc. control cortex rep 1	8.7	36,067,046	36,067,046	94.00	88.01	9.65
Transc. control cortex rep 2	8.7	33,261,134	33,261,134	93.84	87.79	9.78
Transc. trained axons rep 1	9.6	37,890,759	37,890,759	94.16	88.04	9.63
Transc. trained axons rep 2	8.8	39,793,039	39,793,039	94.02	87.81	9.63
Transc. trained cortex rep 1	8.6	31,509,058	31,509,058	93.81	88.15	9.42
Transc. trained cortex rep 2	9.0	31,031,259	31,031,259	95.58	87.72	9.61
YFP_IP control axons rep 1	7.0	39,073,113	39,073,113	94.15	74.32	21.75
YFP_IP control axons rep 2	9.0	32,214,031	32,214,031	94.25	72.90	22.99
YFP_IP control cortex rep 1	8.8	27,039,569	27,039,569	93.57	76.52	19.51
YFP_IP control cortex rep 2	9.3	27,888,237	27,888,237	93.17	73.15	22.23
YFP_IP trained axons rep 1	9.0	27,119,148	27,119,148	92.58	74.22	21.69
YFP_IP trained axons rep 2	8.4	29,286,890	29,286,890	95.23	73.60	22.19
YFP_IP trained cortex rep 1	9.5	30,180,396	30,180,396	94.74	76.00	19.55
YFP_IP trained cortex rep 2	8.9	29,087,509	29,087,509	93.94	74.33	21.60
YFP transc. control axons rep 1	9.5	32,819,895	32,819,895	94.16	88.17	9.33

Table S1. RNA Quality Control Data, cont.

Sample	RIN	Raw reads #1	Raw reads #2	% bases Q>= 30	Uniquely mapped reads %	Multi-mapped reads %
YFP transc. control axons rep 2	9.4	32,118,423	32,118,423	94.29	86.84	10.52
YFP transc. control cortex rep 1	9.6	29,502,761	29,502,761	93.81	87.73	9.71
YFP transc. control cortex rep 2	7.6	30,411,787	30,411,787	93.38	87.43	9.86
YFP transc. trained axons rep 1	9.6	29,436,121	29,436,121	92.82	88.19	9.30
YFP transc. trained axons rep 2	9.1	33,504,177	33,504,177	95.48	87.93	9.49
YFP transc. trained cortex rep 1	9.4	33,113,755	33,113,755	95.15	87.57	9.53
YFP transc. trained cortex rep 2	9.6	31,485,033	31,485,033	94.04	87.87	9.57

RIN: RNA Integrity Number; $Q = -10 \times \log_{10}(p)$ where p=probability of incorrect base call

Supplementary Tables 2-8 are in a separate Excel file

Supplementary Table 2. Results of differential gene expression analysis and subsequent filtering.

Supplementary Table 3. Results of differential gene expression analysis and subsequent filtering, YFP samples.

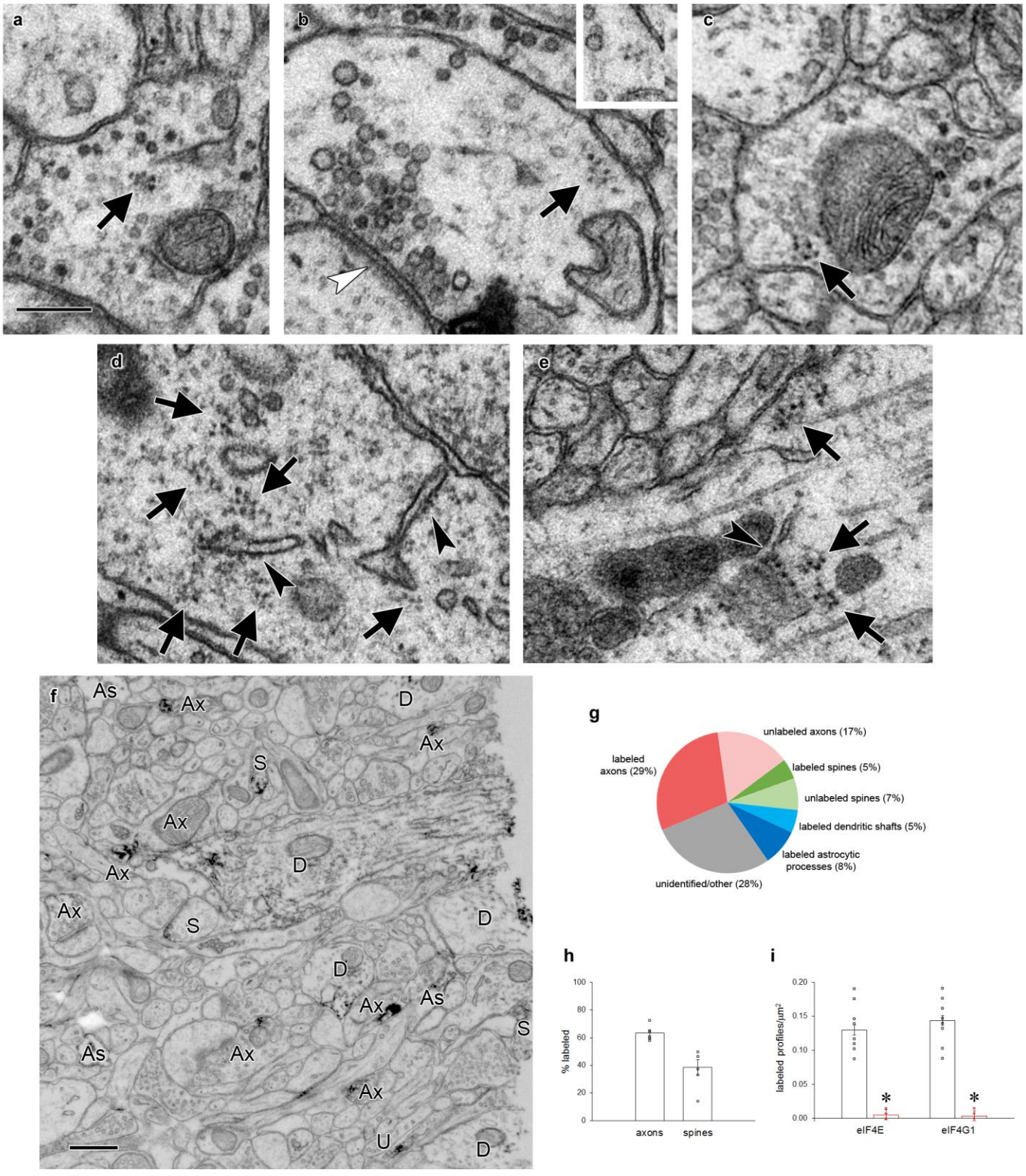
Supplementary Table 4. Results of DAVID enrichment analyses of all axonal genes, cortex-only genes, and genes that were upregulated and downregulated in the axons and cortex.

Supplementary Table 5. Results of ANOVA and post hoc Bonferroni test comparing mean FPKM between experimental groups by learning effect.

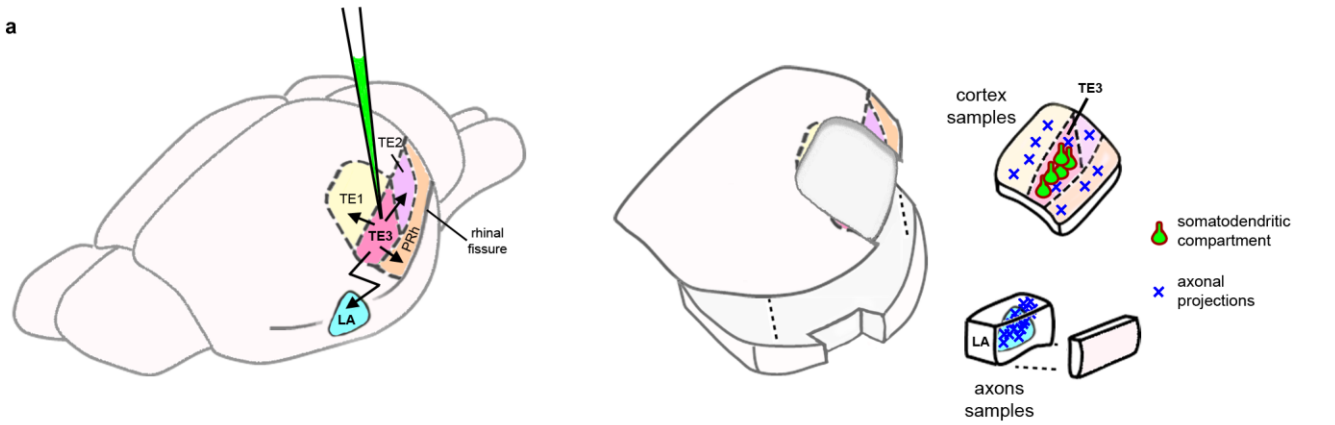
Supplementary Table 6. Results of IPA Upstream Regulator analysis of learning effects in axons and cortex.

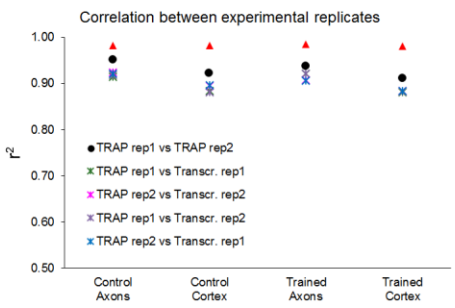
Supplementary Table 7. Results of IPA Functional Annotation analysis of learning effects in axons and cortex.

Supplementary Table 8. Transcript-level FPKM values and results of differential expression analysis.


a
b
c
d
e
f
As
Ax
D
S
g
unlabeled axons (17%)
labeled spines (5%)
unlabeled spines (7%)
labeled dendritic shafts (5%)
labeled astrocytic processes (8%)
unidentified/other (28%)
labeled axons (29%)
h
% labeled
axons
spines
i
labeled profiles/µm²
eIF4E
eIF4G1

Supplementary Figure 1. Polyribosomes and translation factors in axons. a-c) Examples of polyribosomes (arrows) in axonal boutons. Inset in (b) shows the same polyribosome on an adjacent serial section. d-e) Copious polyribosomes (arrows) in a neuronal cell body (d) and a large dendritic shaft (e). Rough endoplasmic reticulum (arrowheads) is visible in both structures. f) Representative field of tissue immunolabeled for eIF4E, with labeled axons (Ax), astrocytic processes (As), dendritic shafts (D), and dendritic spines (S) indicated. Profiles were followed through serial sections to confirm identifications. g) Breakdown of all profiles in a 4μm^2 field of one section near the center of a serial EM volume of tissue immunolabeled for eIF4E. Six series were averaged. 28% of profiles could not be unambiguously identified within the series. h) Percent of axons and spines in a 4μm^2 field that were immunolabeled for eIF4E when followed through series. 100% of dendritic shafts and astrocytic processes contained label.





c

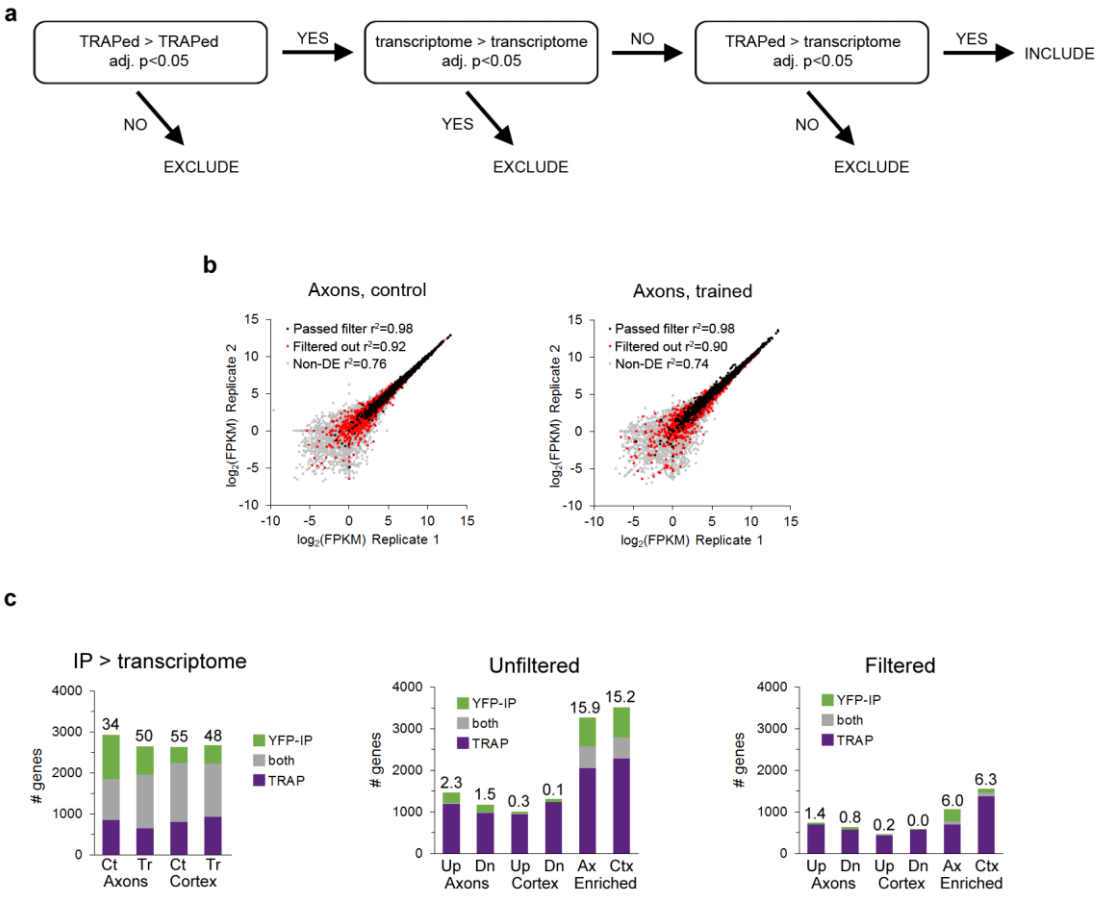
Enriched in Neurons	log2(FoldChange), TRAP vs Tissue Transcriptome			
	Control Axons	Trained Axons	Control Cortex	Trained Cortex
Bcl11a			-0.58	
Zfp238	0.61	0.53	0.61	0.88
Tp53i11	-0.35	-0.44	-0.57	
Dcx			-1.31	
Bcl11b	0.61	0.74	1.16	
Vprbp	0.98			1.54
Nsg1	-0.49			-0.74
Dut		0.71	0.60	
Hirip3		0.66	1.04	
Camta1	0.77	0.65	0.93	1.16
Ahdc1	1.29	1.00	1.40	1.87
Ubqln1	-1.56	-1.61	-1.29	-1.20
Syngap1	1.68	1.72	1.37	1.92
Igsf3	0.91	1.15	0.70	0.72
Nsg2		0.32	0.52	0.26
Rai1	0.82			1.09
Usp29	0.71		1.00	
Dapk1	1.23	0.95	0.93	1.13
Celf4	-0.23	-0.19		
Dgkg	0.77		0.75	
Ss18l1		-1.41	-1.44	-1.47
Tyms		-1.71		
Brsk1	1.46	0.90		1.55
Afap1		1.43		
Dlg3			-0.84	
Sugp2	0.84		0.41	1.33
Pbx1	-1.13		-1.58	-1.35
Ep400	1.44	1.48	1.29	1.33
Map9	0.31	0.91	1.37	0.94
Calb2	-1.25	-0.86	-0.78	-1.15
Ssh2	1.62	1.05		0.96
Phc2		-0.41		-0.54
Scg2		0.63	0.83	0.43
Ociad2	-1.71		-1.43	-1.05
Sptbn2	4.24	2.32	1.60	3.66
Serpini1	-0.29	-0.38		
Baz2b	1.02	0.54	1.20	1.21
Cpne5	1.27	0.75	0.77	1.14
Kif5c	0.71	0.58	0.30	0.55
Elavl3	0.58	0.83	1.02	1.08
Nell2	0.25	0.43	0.45	0.41
Ttc26			-1.84	
Chd5	1.75	2.18	2.49	2.73
Whsc1	-1.29	-1.08		-0.98
Arhgap39	1.50			
Mtss1		0.80	0.97	0.71
Chd8	1.48	1.63	2.09	1.45
Fnbp1l	0.89	1.24	1.19	1.05
Ccsap		0.84	1.35	
Kdm5b	1.68	0.79	0.94	1.19

Depleted in Neurons	log2(FoldChange), TRAP vs Tissue Transcriptome			
	Control Axons	Trained Axons	Control Cortex	Trained Cortex
S100a4		1.30	1.39	1.23
Sec14l2				-0.73
Myo9b	1.18			
Itgb8	0.82	1.75	0.84	1.22
Hebp1	-0.70		-1.36	-0.75
Padi2	0.77			0.91
Tns3	0.87			1.04
Arhgap5			0.97	
Pggt1b		-1.46	-1.35	-2.18
Prex1			-0.68	
Gys1	0.96			
Cnp	0.51	-0.19		
Gatm	-0.61	-1.39	-1.54	-0.79
Sumf1	-0.78			-1.09
Acox3				-1.41
Abhd4		-1.60	-1.28	-0.64
Erap1			-1.72	
Sept10		-1.43		
Cers2	-1.11	-1.20	-1.50	-1.40
Tor1aip2	-0.94	-1.29		-0.72
Pld1			-1.15	
Akr1b10	-1.25			-2.39
Qk		-0.54	-0.61	-1.14
Parvb	-1.72	-1.47	-1.50	-1.55
Cluh	0.84		0.47	0.89
Stom				-1.52
Sirpa			0.44	
Camk1				-0.28
Csad	0.64		1.12	0.71
Emb				-0.83
Pla2g7	-1.35			
Aldoa	-0.25	-0.19	-0.43	-0.52
Rac1	-0.73	-1.35	-1.55	-1.27
Tbc1d9b	0.72			
Pxn			-0.92	
Mgat1		0.82	1.19	
Xpot		-0.69		
Sh3glb1	-1.09			-0.69
Sri	-1.08		-0.69	-0.62
Arl8b		-0.28	-0.39	-0.25
Marc2		0.59		
Gfpt1		1.17	1.04	
Snx4	-0.70	-0.47		
Ilvbl	-0.56			-0.55
Snx3	-0.31	0.23		
Ube2j1	-2.44	-1.83	-2.43	-2.51
Papss1	0.66			0.54
Anxa6			-0.78	-0.65
Aprt	0.31	0.75	0.77	0.53
Arf1	-0.83	-1.07	-2.11	-1.70

log2(FoldChange)
2
0
-2

Supplementary Figure 2. Collection of TRAP samples. a) Left: Illustration of LV-CMV-eYFP-L10a injection into cortical area TE3, showing TE3 projections to cortical areas TE1, TE2, and perirhinal (PRh), and the lateral amygdala (LA). Right: Illustration of tissue sampling for TRAP. After separating the hemispheres and bisecting along the rhinal fissure, cortex samples were collected by dissecting wide margins around TE3 so that portions of adjacent cortical areas and the underlying white matter

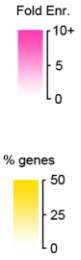
were included. A separate block was dissected from the ventral half (the “axons” sample), containing the LA, along with the immediately adjacent small area of caudate that also receives projections from TE3. The adjacent area of cortex was removed to ensure that these samples did not contain any stray pieces of perirhinal cortex that could contain cortico-cortical axons. Cortical divisions and projection patterns adapted from references 25-27. b) Correlation coefficients of $\log_2$(FPKM) between experimental replicates, calculated from all raw data. c) The top genes in the proteome of adult mouse cortex identified as enriched (left) or depleted (right) in neurons versus other cell types, sorted by magnitude of enrichment [44]. The top 50 genes that were also significantly enriched or depleted in our TRAPed samples versus the tissue transcriptome are shown, with the normalized magnitude of change. Significance was defined as an adjusted p value of <0.05. Neuron-enriched genes were mostly enriched in TRAPed samples (36 of 50), while neuron-depleted genes were depleted from TRAP samples (34 of 50).



Supplementary Figure 3. Filtering of DGE results. a) Strategy for removing false positives from results of differential gene expression analysis. b) FPKM values of TRAPed genes from axons in experimental replicates of the control (left) and trained (right) groups. All genes defined as axonal that passed the filtering procedure are indicated with black markers, axonal genes that were removed by filtering with red, and genes that were not axonal in gray. c) Overlap between DGE results in the TRAP and YFP-IP experiments. Left: genes enriched in the TRAP and YFP IP samples versus the transcriptome for all four experimental conditions. Numbers above the bars indicate percent overlap. Center, right: Overlap between genes regulated in axons and cortex (Up, upregulated; Dn, downregulated) or enriched in the axons versus cortex in the unfiltered data (center) and filtered data (right).

a

	Top terms by p-value	Fold Enrichment		p-value		% genes	
		Filtered	Unfilt.	Filtered	Unfilt.	Filtered	Unfilt.
Filtered Axons	Poly(A) RNA binding	3.08	2.03	1.4E-62	7.7E-40	17.8	11.6
	Mitochondrion	2.50	1.89	1.9E-50	1.5E-43	20.3	15.1
	Ribosome	6.12	3.05	7.1E-49	1.2E-22	6.2	3.0
	Oxidative phosphorylation	5.94	3.31	3.1E-38	9.1E-24	5.1	2.7
	Extracellular exosome	1.91	1.70	1.2E-36	1.4E-49	25.6	22.4
	Myelin sheath	5.59	3.62	2.8E-36	5.9E-33	5.4	3.4
	Cytoplasm	1.56	1.45	3.4E-36	2.1E-52	41.3	37.9
	Parkinson's disease	5.48	3.26	2.7E-34	9.6E-24	4.9	2.8
	Huntington's disease	4.61	2.56	2.1E-32	5.0E-16	5.5	2.9
	Alzheimer's disease	4.70	2.78	3.9E-30	1.7E-18	5.1	2.9
Unfiltered Axons	Cytoplasm	1.56	1.45	3.4E-36	2.1E-52	41.3	37.9
	Extracellular exosome	1.91	1.70	1.2E-36	1.4E-49	25.6	22.4
	Protein binding	2.05	1.91	1.4E-26	9.4E-46	16.6	15.3
	Mitochondrion	2.50	1.89	1.9E-50	1.5E-43	20.3	15.1
	Poly(A) RNA binding	3.08	2.03	1.4E-62	7.7E-40	17.8	11.6
	Myelin sheath	5.59	3.62	2.8E-36	5.9E-33	5.4	3.4
	Cytosol	1.78	1.70	1.7E-15	7.9E-28	14.3	13.5
	Membrane	1.68	1.56	1.6E-17	2.7E-26	18.8	17.1
	Oxidative phosphorylation	5.94	3.31	3.1E-38	9.1E-24	5.1	2.7
	Parkinson's disease	5.48	3.26	2.7E-34	9.6E-24	4.9	2.8



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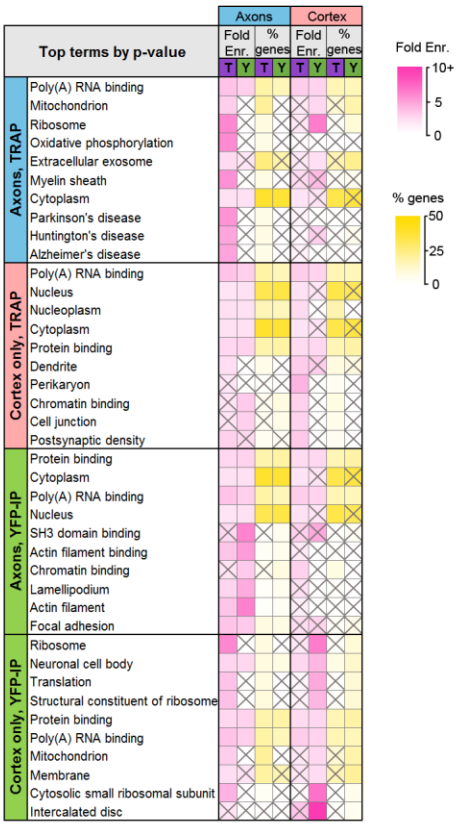
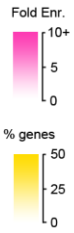


Supplementary Figure 4. Comparison of TRAP and YFP-IP experiments. a) Top GO and KEGG Pathway terms enriched in the filtered and unfiltered sets of axonal genes, sorted by Benjamini-Hochberg adjusted p-value. b) Top GO Terms and KEGG pathways in axonal and cortex-only translatomes in TRAP and YFP-IP samples, sorted by Benjamini-Hochberg adjusted p-value. Gray X's indicate effects that were not significant (adjusted p-value >0.05).

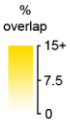
a

Significantly Enriched Terms	Fold Enrich.		% of genes	
	Axons	Cortex	Axons	Cortex
Presynaptic compartment				
Myelin sheath	5.6		5.4	
Axon	2.4		4.5	
Axon cytoplasm	4.5		0.8	
Axonal growth cone	3.9		0.6	
Synaptic vesicle	2.4		1.6	
Metabolism/mitochondrial				
Mitochondrion	2.5		20.3	
Oxidative phosphorylation	5.9		5.1	
Metabolic pathways	1.5		11.6	
Citrate cycle (TCA cycle)	5.4		1.0	
RNA Processing/Translation				
Catalytic step 2 spliceosome	3.7	3.8	1.8	1.7
Poly(A) RNA binding	3.1	2.6	17.8	14.8
Spliceosome	3.1		2.5	
Ribosome	6.1		6.2	
Translation	3.1		6.0	
Golgi apparatus	1.4		5.9	
Cytoskeleton/transport/cell adhesion				
Cell junction		2.3		4.5
Microtubule	2.9		3.4	
Cytoskeleton	2.5		3.0	
Actin binding	2.4		3.1	
Motor activity	4.0		1.1	
Cadherin binding involved in cell-cell adhesion	2.1		2.3	
Nucleus/transcription				
Chromatin binding		2.4		5.4
Nucleus	1.4	1.5	34.4	36.0
DNA-directed RNA polymerase II, core complex	4.7		0.5	
Cell body				
Perikaryon		3.8		2.8
Perinuclear region of cytoplasm	1.8	1.9	5.9	5.8
Neuronal cell body	2.2	2.0	6.0	5.2
Postsynaptic compartment				
Dendrite	1.7	2.6	4.2	6.1
Dendrite membrane		8.4		1.0
Postsynaptic density	2.4	2.8	2.9	3.2
Postsynaptic membrane		2.7		2.6
Dendritic spine	2.6		1.9	
Other				
Zinc ion binding		1.7		9.0
Extracellular exosome	1.9		25.6	
Cytoplasm	1.6	1.4	41.3	35.8
Parkinson's disease	5.5		4.9	
Huntington's disease	4.6		5.5	
Alzheimer's disease	4.7		5.1	
Membrane	1.7		18.8	
Proteasome complex	4.1		1.2	
Calmodulin binding	2.6		2.1	
Positive regulation of GTPase activity	1.8		3.6	
Non-alcoholic fatty liver disease (NAFLD)	4.0		3.8	

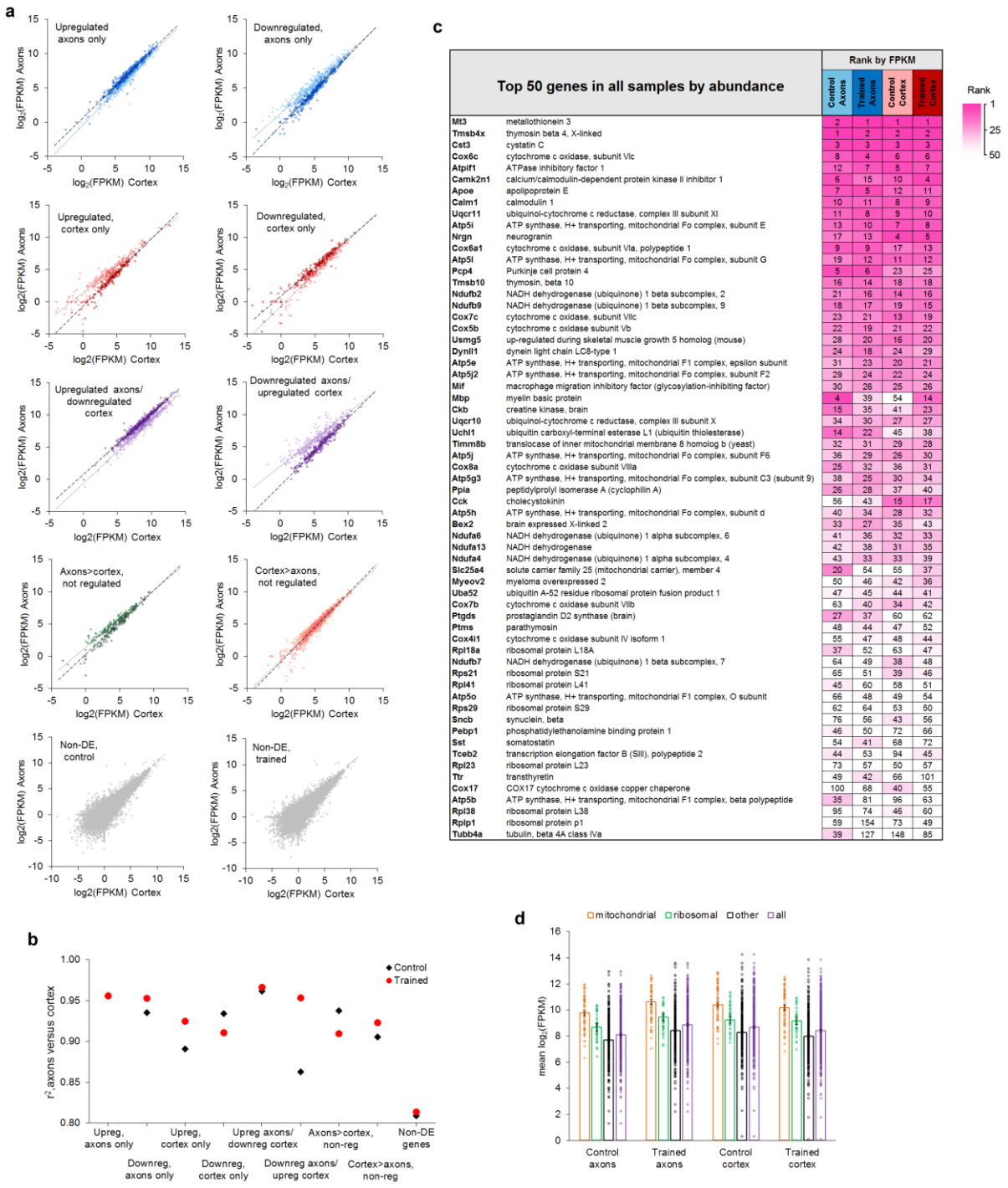


b

	Overlap with published axonal translatome/transcriptomes	% overlap		# of genes	
		Ax	Ct	Ax	Ct
Axonal transl.	Adult RGCs, *in vivo*[20]	4.1	2.3	77	26
	Immature RGCs, *in vivo*[20]	14.1	4.9	460	139
Axonal transcrip.	DRG, mature cultures[17]	13.0	4.5	424	126
	DRG, developing cultures[17]	17.4	5.7	520	147
	Cortex, mature cultures[19]	8.4	2.0	137	19
	Cortex, mature cultures, upreg. after injury[19]	1.6	1.0	31	12
	Cortex, mature cultures, downreg. after injury[19]	4.9	2.2	87	24
	DRG, injured, developing cultures[18]	6.7	1.5	112	13
	Motor neurons, developing cultures[46]	4.5	5.2	64	35
Neuropil transcrip.	Adult CA1, acute slices[8]	11.5	5.8	415	177
	Adult CA1, *in vivo*[9]	1.5	0.9	23	7
	Cultured CA1[7]	1.0	0.8	16	7
	Juvenile cortical synaptoneurosomes (transl.)[10]	3.7	1.7	53	12



Supplementary Figure 5. Composition of the axonal translatome. a) Groups of related terms enriched in axonal, cortex-only, or both gene sets. Text color indicates higher enrichment in axons (blue) or cortex (red). Only significant effects (adjusted p-value <0.05) are shown. b) Overlap (% intersection/union) between the axonal and cortex-only and published translatomes and transcriptomes in references 8-10 and 16-19, and number of overlapping genes.



Supplementary Figure 6. Relative abundance of genes in axons and cortex. a) Plots of log2(FPKM) in cortex versus axons in control (light markers) and trained (dark markers) groups, grouped by learning effects. b) Correlation coefficients between $\log_2$(FPKM) in cortex and axons for each learning effect. c) 63 genes representing the top 50 genes from each of the four groups, sorted by average rank. d) Mean FPKM of genes upregulated in axons and downregulated in cortex after learning, grouped into mitochondrial respiration (n=55), ribosomal proteins (n=39), the remainder (n=294), and the full gene set (n=388). Error bars= s.e.m.

a

	Top Regulated IPA Functional Annotations	Activation z-score		# of genes		p-value	
		Axons	Cortex	Axons	Cortex	Axons	Cortex
Axons	formation of cellular protrusions	-1.529	1.229	93	67	3.3E-06	6.4E-04
	neuritogenesis	-1.145	1.104	92	66	4.3E-06	8.6E-04
	microtubule dynamics	-1.634	0.941	102	73	5.8E-06	1.3E-03
	morphology of axons			24	16	1.3E-05	2.1E-03
	potentiation of synapse	-2.913	2.475	43	29	3.8E-05	5.8E-03
	abnormal morphology of neurites			30	23	4.2E-05	4.1E-04
	development of neurons	-0.955	0.531	101	74	4.5E-05	2.2E-03
	quantity of neurofilaments	-0.577	0.577	4	4	4.6E-05	1.6E-05
	axonal transport	-2.425		7		5.2E-05	
	long-term potentiation	-3.148	2.767	42	28	6.3E-05	9.1E-03
Cortex	quantity of neurofilaments	-0.577	0.577	4	4	4.6E-05	1.6E-05
	size of axons			4	4	2.2E-04	7.8E-05
	plasticity of neuronal synapse			8	7	1.6E-04	2.2E-04
	plasticity of synapse	-1.664	1.026	23	20	4.1E-04	2.3E-04
	morphology of dendrites			15	16	8.8E-03	2.4E-04
	cell death of pyramidal neurons		1.372	4	6	3.8E-02	3.0E-04
	size of neurons			17	15	6.5E-04	3.6E-04
	abnormal morphology of neurites			30	23	4.2E-05	4.1E-04
	morphology of neurites			38	30	1.3E-04	5.0E-04
	long term depression	-1.262	0.686	17	18	2.1E-02	6.3E-04

Activation z-score: 3, 0, -3

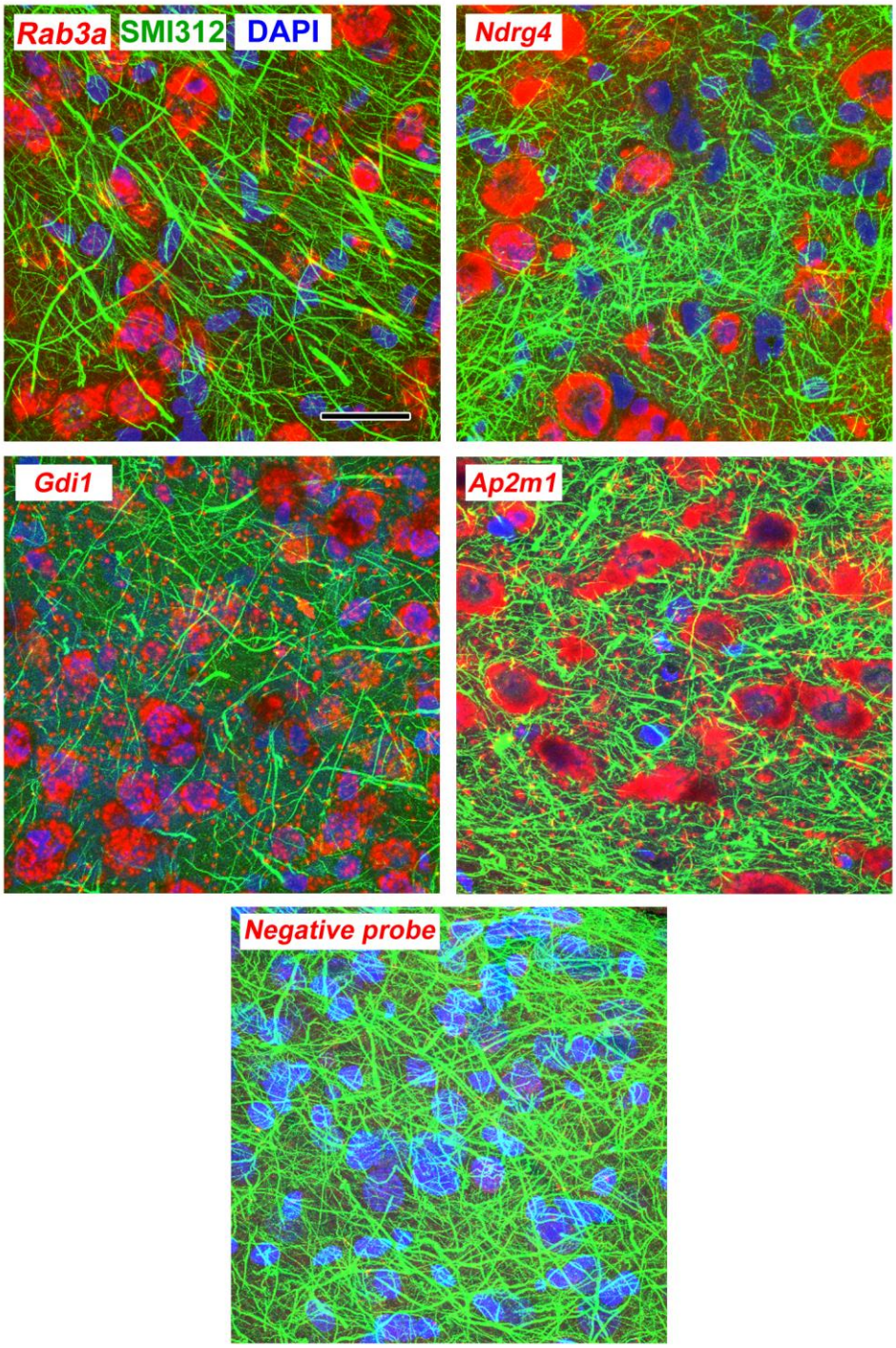
b

	Overlap with published axonal translatome/transcriptomes	% overlap		# of genes		log_2(up/down)
		Upreg Axons	Downreg Axons	Upreg Axons	Downreg Axons	
Translatomes	Adult RGCs, *in vivo*[20]	2.7	3.7	30	37	-0.30
	Immature RGCs, *in vivo*[20]	7.1	8.4	195	220	-0.17
Transcriptomes	DRG, mature cultures[17]	10.3	4.6	271	121	1.16
	DRG, developing cultures[17]	13.8	6.3	331	155	1.09
	Cortex, mature cultures[19]	12.9	2.4	113	20	2.50
	Cortex, mature cultures, upreg. after injury[19]	1.3	1.2	15	13	0.21
	Cortex, mature cultures, downreg. after injury[19]	5.5	2.9	57	27	1.08
	DRG, injured, developing cultures[18]	11.6	2.4	93	18	2.37
	Motor neurons, developing cultures[46]	4.1	1.1	47	12	1.97

% overlap: 15, 7.5, 0

log_2(up/down): 1, 0, -1

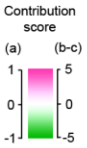
Supplementary Figure 7. a) Functional annotations significantly regulated by learning in the axons and cortex. b) Overlap between genes regulated in axons and published translatomes and transcriptomes in references (16-19).



Supplementary Figure 8. Maximum intensity projections through 3µm (10 confocal images with a 0.3µm z-step size) of lateral amygdala showing FISH labeling and immunolabeling for neurofilaments. Scale = 20 µm.

a

Gene	transcript	log_2(fold change)	adj. p value	contrib. score
		Trained vs control axons		
Snx27	NM_152847	-0.99	0.021	5.99
	NM_001110151	0.82	0.401	-4.99
Speg	NM_001108802	-1.43	0.009	1.14
	NM_012905	0.17	0.781	-0.14
Gng2	NM_031754	0.80	0.033	2.55
	NM_001257349	-0.49	0.523	-1.55
		Control cortex vs axons		
Gria2	NM_001083811	1.53	0.001	3.78
	NM_017261	-1.12	0.026	-2.78



b

Upregulated axons/ downregulated cortex		contribution score			adj. p value	
gene	transcript	axons	cortex	axons-cortex	axons	cortex
Apoe	NM_001270681	0.00	0.01	-0.01	0.876	0.942
	NM_001270682	0.00	0.00	0.00	0.704	0.957
	NM_001270683	0.00	0.00	0.00	1.000	1.000
	NM_001270684	0.75	0.73	0.02	0.031	0.730
	NM_138828	0.25	0.26	-0.01	0.359	0.863
Arpp19	NM_031660	0.81	0.76	0.05	0.729	0.781
	NM_031660_1	0.19	0.24	-0.05	0.878	0.844
Calm2	**NM_017326**	0.32	0.27	0.05	0.002	0.018
	NM_017326_1	0.68	0.73	-0.05	0.002	0.004
Caly	NM_001190399	0.37	0.13	0.24	0.588	0.983
	NM_138915	0.63	0.87	-0.24	0.115	0.837
Chchd10	NM_001007008	1.00	1.00	0.00	0.004	0.327
	NM_001007008_1	0.00	0.00	0.00	1.000	1.000
Dpy30	NM_001170545	1.00	1.00	0.00	0.005	0.550
	NM_173117	0.00	0.00	0.00	1.000	1.000
Fau	NM_001012739	0.59	1.04	-0.46	0.432	0.499
	NM_001160231	0.44	-0.17	0.61	0.462	0.894
	NM_001160232	-0.03	0.13	-0.15	0.933	0.781
Fkbp2	NM_001134428	0.59	0.65	-0.06	0.246	0.583
	NM_001134429	0.41	0.35	0.06	0.286	0.680
Gpx4	NM_001039849	0.00	-0.12	0.11	0.973	0.815
	NM_017165	1.00	1.12	-0.11	0.335	0.716
Hcfc1r1	NM_001100492	1.04	1.03	0.01	0.074	0.484
	NM_001185047	-0.04	-0.03	-0.01	0.776	0.945
Hnrnpa3	**NM_001111294**	1.05	0.67	0.38	0.010	0.011
	NM_001111295	-0.02	0.00	-0.02	1.000	1.000
	NM_198132	-0.03	0.33	-0.36	0.976	0.680
Hnrnpd	NM_001082539	0.72	0.54	0.18	0.573	0.591
	NM_001082540	0.71	0.27	0.44	0.409	0.720
	NM_001082541	-0.29	0.09	-0.39	0.745	0.867
	NM_024404	-0.13	0.10	-0.23	0.907	0.943
Klc1	NM_001081972	0.95	1.07	-0.12	0.756	0.688
	NM_001081973	-0.09	-0.03	-0.07	0.930	0.908
	NM_001081974	0.14	-0.04	0.19	0.972	0.988
LOC100233176	NM_001142941	0.71	2.06	-1.35	0.672	0.336
	NM_001142942	0.29	-1.06	1.35	0.801	0.481
Mif	NM_031051	1.00	1.00	0.00	0.002	0.351
	NM_031051_1	0.00	0.00	0.00	1.000	1.000
Mrps21	NM_001126094	-0.01	-0.02	0.01	0.886	0.769
	NM_001287116	1.01	1.02	-0.01	0.156	0.680
Naca	NM_001105939	0.04	0.12	-0.08	0.772	0.520
	NM_001198562	0.96	0.88	0.09	0.002	0.098
	NM_001198580	0.00	0.00	0.00	1.000	1.000
Ndufv3	NM_001101011	0.87	0.82	0.05	0.185	0.697
	NM_022607	0.13	0.18	-0.05	0.468	0.634
Oxr1	NM_001197332	0.82	1.13	-0.31	0.086	0.009
	NM_001197907	0.17	-0.12	0.29	0.347	0.609
	NM_057153	0.01	-0.01	0.03	0.903	0.941
Park7	NM_001277249	0.00	0.00	0.00	1.000	1.000
	NM_001277250	0.23	0.07	0.16	0.219	0.901
	NM_001277251	0.00	0.00	0.00	1.000	1.000
	NM_001277252	0.74	0.83	-0.10	0.014	0.337
	NM_001277253	0.02	0.10	-0.07	0.896	0.781
	NM_057143	0.01	-0.01	0.01	0.691	0.649
Pcp4	NM_001270538	0.00	0.00	0.00	0.960	0.601
	NM_013002	1.00	1.00	0.00	0.004	0.337
Pfdn6	NM_001164718	-0.01	0.67	-0.68	0.996	0.738
	NM_212506	1.01	0.33	0.68	0.486	0.836
Pnkd	NM_001134750	-0.01	-0.06	0.05	0.924	0.800
	NM_001134751	0.05	0.17	-0.12	0.800	0.695
	NM_001134753	0.96	0.89	0.07	0.357	0.591
Rpl35a	NM_021264	0.00	0.00	0.00	1.000	1.000
	NM_021264_1	0.85	0.92	-0.06	0.275	0.651
	NM_021264_2	0.08	0.04	0.03	0.636	0.907
	NM_021264_3	0.07	0.04	0.03	0.644	0.888
	NM_021264_4	0.00	0.00	0.00	1.000	1.000
Rpl38	NM_001077592	0.00	0.00	0.00	0.761	0.843
	NM_001077592_1	0.00	0.00	0.00	0.836	0.856
	NM_001077592_2	1.00	1.00	0.00	0.073	0.155
Rps13	NM_130432	0.99	0.99	0.01	0.011	0.573
	NM_130432_1	0.01	0.01	-0.01	0.844	0.784
Rps14	NM_022672	0.97	0.96	0.01	0.055	0.707
	NM_022672_1	0.03	0.04	-0.01	0.765	0.924
Rps27a	NM_001305443	0.33	0.27	0.06	0.188	0.602
	NM_031113	0.67	0.73	-0.06	0.134	0.428
Spint2	NM_001082549	0.04	-0.17	0.21	0.881	0.638
	NM_199087	0.96	1.17	-0.21	0.216	0.645
Tac1	NM_001124768	0.70	0.67	0.03	0.234	0.568
	NM_001124769	-0.02	-0.05	0.03	0.956	0.967
	NM_001124770	0.01	0.20	-0.19	0.976	0.827
	NM_012666	0.31	0.18	0.13	0.515	0.835
Tceb1	NM_001270561	-0.02	0.00	-0.02	1.000	1.000
	NM_001270562	0.94	0.71	0.23	0.016	0.250
	NM_001270563	0.05	0.11	-0.07	0.849	0.669
	NM_022593	0.02	0.17	-0.15	0.924	0.645
Tmem14c	NM_001135169	1.10	1.20	-0.10	0.144	0.487
	NM_134395	-0.10	-0.20	0.10	0.738	0.782

Upregulated axons/ downregulated cortex, cont.		contribution score			adj. p value	
gene	transcript	axons	cortex	axons-cortex	axons	cortex
Apoe	NM_001270681	0.00	0.01	-0.01	0.876	0.942
	NM_001270682	0.00	0.00	0.00	0.704	0.957
	NM_001270683	0.00	0.00	0.00	1.000	1.000
	NM_001270684	0.75	0.73	0.02	0.031	0.730
	NM_138828	0.25	0.26	-0.01	0.359	0.863
Tpm1	NM_001034068	0.31	0.24	0.07	0.599	0.830
	NM_001034069	0.01	-0.03	0.03	0.842	0.704
	NM_001034070	0.00	0.00	0.00	1.000	1.000
	NM_001034071	0.03	0.05	-0.02	0.714	0.639
	NM_001034072	-0.08	0.13	-0.21	0.875	0.880
	NM_001034073	0.19	0.26	-0.07	0.561	0.678
	NM_001034074	-0.06	0.08	-0.14	0.671	0.510
	NM_001034075	0.20	-0.06	0.26	0.548	0.937
	NM_001301336	-0.04	-0.14	0.09	0.774	0.680
	NM_001301342	-0.03	-0.01	-0.02	0.860	0.956
	NM_001301736	0.46	0.06	0.40	0.715	0.990
	NM_019131	0.01	0.40	-0.39	0.988	0.928
Uqcr11	NM_001126097	0.95	0.98	-0.03	0.004	0.241
	NM_001287109	0.05	0.02	0.03	0.779	0.951

c

Downregulated axons/ upregulated cortex		contribution score			adj. p value	
gene	transcript	axons	cortex	axons-cortex	axons	cortex
Akap2	NM_001011974	0.21	0.28	-0.08	0.135	0.724
	NM_001309260	0.79	0.72	0.08	0.002	0.137
Begain	NM_001111115	0.07	0.00	0.07	0.833	1.000
	NM_024163	0.93	1.00	-0.07	0.121	0.004
Camk2b	NM_001042354	-0.01	0.01	-0.02	1.000	0.596
	NM_001042356	0.19	-0.17	0.37	0.809	0.834
	NM_021739	0.81	1.16	-0.35	0.428	0.057
Csnk1g2	**NM_001033870**	0.83	0.77	0.06	0.022	0.004
	NM_023102	0.17	0.23	-0.06	0.503	0.283
Dlgap1	**NM_001304287**	1.01	0.91	0.11	0.005	0.009
	NM_022946	-0.01	0.09	-0.11	0.931	0.728
Dlgap3	NM_001276304	0.07	0.12	-0.04	0.753	0.724
	NM_001301876	0.93	0.88	0.04	0.028	0.199
Iqsec2	NM_001277386	-0.01	-0.04	0.03	1.000	1.000
	NM_001277425	1.01	1.04	-0.03	0.054	0.129
Kdm5a	**NM_001277177**	1.00	1.00	0.00	0.006	0.006
	NM_001277178	0.00	0.00	0.00	1.000	1.000
Mbp	NM_001025289	0.00	0.00	0.00	0.909	0.976
	NM_001025291	0.04	0.02	0.02	0.547	0.747
	NM_001025292	0.15	0.20	-0.05	0.161	0.033
	NM_001025293	0.13	0.11	0.02	0.070	0.549
	NM_001025294	0.02	0.02	0.00	0.817	0.798
	NM_017026	0.66	0.65	0.02	0.002	0.004
Mprip	NM_001034022	0.95	0.92	0.02	0.002	0.004
	NM_053814	0.05	0.08	-0.02	0.186	0.114
Nexn	NM_139230	0.86	0.94	-0.09	0.002	0.231
	NM_139231	0.14	0.06	0.09	0.468	0.936
Nsmf	NM_001270626	0.57	0.00	0.57	0.543	1.000
	NM_001270627	-0.17	0.34	-0.51	0.757	0.758
	NM_001270628	-0.10	0.08	-0.18	0.644	0.744
	NM_057190	0.46	0.04	0.43	0.330	0.803
	NR_073057	0.24	0.54	-0.30	0.699	0.765
Pde2a	NM_001143847	0.10	-0.01	0.11	0.633	0.990
	NM_001270604	0.65	0.63	0.02	0.481	0.679
	NM_031079	0.25	0.38	-0.13	0.821	0.805
Pip5k1c	NM_001009967	0.61	0.53	0.08	0.600	0.184
	NM_001033970	0.39	0.47	-0.08	0.715	0.373
Plec	**NM_001164296**	0.84	0.85	-0.01	0.002	0.004
	NM_001164297	0.01	0.12	-0.10	1.000	0.708
	NM_001164298	0.04	0.01	0.02	1.000	1.000
	NM_001164299	0.00	0.00	0.00	1.000	1.000
	NM_001164302	0.00	0.00	0.00	1.000	1.000
	NM_001164303	0.00	0.00	0.00	1.000	1.000
	NM_001164304	0.07	0.00	0.07	0.739	1.000
	NM_001164305	0.04	0.00	0.04	0.710	1.000
	NM_001164307	0.00	0.00	0.00	1.000	1.000
	NM_001164308	0.00	0.02	-0.02	1.000	1.000
	NM_022401	0.00	0.00	0.00	1.000	1.000
Prkcb	NM_001172305	0.21	0.37	-0.15	0.726	0.520
	NM_012713	0.79	0.63	0.15	0.050	0.091
Rasgrf1	NM_001105753	0.00	-0.01	0.01	1.000	0.822
	NM_001170531	1.00	1.01	-0.01	0.005	0.070
Ryr2	**NM_001191043**	0.97	0.94	0.03	0.005	0.004
	NM_032078	0.03	0.06	-0.03	1.000	0.720
Shank2	NM_001004133	0.47	0.84	-0.37	0.140	0.487
	NM_133440	0.01	0.04	-0.04	1.000	1.000
	NM_133441	0.14	0.23	-0.08	0.515	0.565
	NM_201350	0.38	-0.11	0.49	0.171	0.909
Slc25a3	NM_001270788	0.00	0.00	0.00	0.944	1.000
	NM_139100	1.00	1.00	0.00	0.018	0.004

Supplementary Figure 9. Transcript-level analysis. The contribution score (change in FPKM transcript/change in FPKM gene) indicates the effect of learning on a transcript relative to the net effect

on all transcripts of the same gene, with a negative score indicating differences in opposite directions between the transcript and gene. Adjusted p-values for each transcript are highlighted at <0.05. a) Three transcripts were found to be regulated by learning in the axons that were not differentially expressed at the gene level. In each case, a second transcript was affected non-significantly in the opposite direction. The two transcripts of *Gria2* were differently distributed in the control group, with one enriched in axons and the other in cortex. b-c) Genes regulated in both axons and cortex (b; upregulated in axons/downregulated in cortex, c; downregulated in axons/upregulated in cortex) with multiple transcripts in the dataset. The difference between the score in the axons and cortex ("axons – cortex") indicates the degree of asymmetry, with positive numbers indicating transcripts which were affected proportionally more in the axons than cortex. Values near zero indicate transcripts that were similarly affected in both areas. Transcripts with significant effects in both areas are shown in bold type.