Supporting Information

Bestman and Cline 10.1073/pnas.0806296105

SI Methods

Electroporation Settings. Electroporation for transfecting cells with plasmid DNA: Patch pipette with ≈1-μm tip diameter containing 1- to 4-μg/μl plasmid DNA. Stimulus settings: 500-ms train, 1-ms pulse, 200 Hz at 1 to 2 μA.

Electroporation for Electrophysiology. Using a pulled micropipette, the brain ventricle was filled plasmid (1–4 μg/μl) followed by stimulation with platinum plate electrodes (5 pulses, 1.6-ms duration, 50 V).

Electroporation for Morpholino and Plasmid Tranfection. A small volume (<1 μl) of plasmid encoding eGFP driven by UAS-Gal4 (1–4 μg/μl) (1) and 500-μM morpholino solution was injected into the ventricle or into the points tectum itself, followed by stimulation with platinum-plate electrodes (three pulses, 1.6-ms duration, 35 V).

Morpholino Oligonucleotide Sequences. CONTROL-MO (a five-base mismatch): GCGAAATTCAATTTGAATCCAATGG; CPEB-MO: GCCAAATTGAATTTCAATGCAATCG.

Saline Composition. Extracellular saline for AMPA mEPSC recordings contains (in mM): 115 NaCl, 4 KCl, 3 $CaCl_2$, 3 Mg Cl_2, 5 Hepes, 10 glucose, 0.01 glycine with 0.1 picrotoxin, and 0.001 TTX.

Internal solution for AMPA mEPSC recordings contains (in mM): 80 Cesium methanesulfonate, 5 Mg Cl_2, 20 tetraethylammonium, 10 EGTA, 20 Hepes, 2 ATP, and 0.3 GTP, (pH 7.2 with CsOH and osmolarity, 255 mOsm).

Internal saline for *in vivo* recordings contains (in mM): 110 K-gluconate, 8 KCl, 5 NaCl, 1.5 MgCl, 20 Hepes, 0.5 EGTA, 4 ATP, and 0.3 GTP, pH 7.2 with KOH (osmolarity, 255 mOsm).

Visual Stimulation Protocol. The visual stimulus protocol (controlled by a Master 8 stimulator, A.M.P.I.) consisted of: a light adaptation period, 100-s long train of 2.5-ms pulses at 200 Hz (because this fast flicker is not perceptible, the effective intensity equals 50% of the stimulus intensity); the light stimulus, 100 s of 2.5-s pulse repeated at 0.2 Hz; and 70 s of darkness. During the final 70-s dark period, the cell access and patch quality were assessed so that the visual stimulus protocol could be immediately repeated.

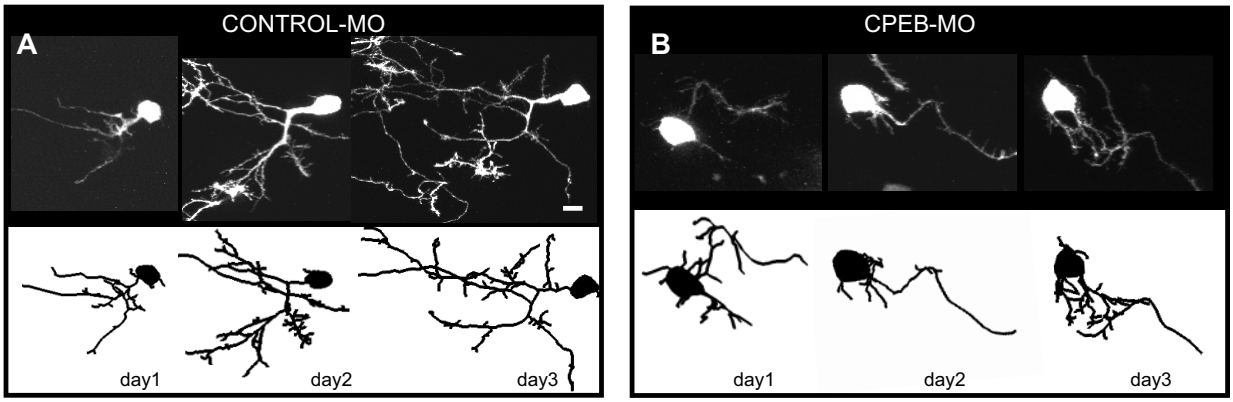
Immunohistochemistry, Microscopy, and Analysis. Two days after electroporation, tadpoles were anesthetized, the skin and membranes around the brain were dissected away, and tadpoles were placed in cold, freshly made 4% paraformaldehyde (Sigma; made in 0.1 M phosphate buffer). The penetration of the fixative was aided by a brief (10–15 s) exposure to 750 W microwave. After fixing for an additional 2 h at room temperature, tadpoles were rinsed in 0.1 phosphate buffer and placed in 30% sucrose where they were stored at 4 °C until cryosectioning (25-μm coronal sections placed on glass slides). All sections were processed in parallel with identical reagents. A standard immunohistochemical protocol was used where the tissue was rinsed in 0.3% Triton X-100 in 0.1 M phosphate buffer and blocked with 5% normal goat serum. The tPA antibody (mouse monoclonal, 2A153; AbCam) was diluted 1:100 in 0.03% Triton X-100 and the sections were incubated 24 h at 4 °C. The sections were rinsed and incubated for 1 h at room temperature in the secondary antibody, Alexa Fluor 633 goat anti-mouse IgG (Molecular Probes), diluted 1:400. After the final rinses in 0.1 M phosphate buffer, the sections were mounted in VectaShield mounting medium with propidium iodide (Vector Laboratories) and stored at −20 °C until imaged.

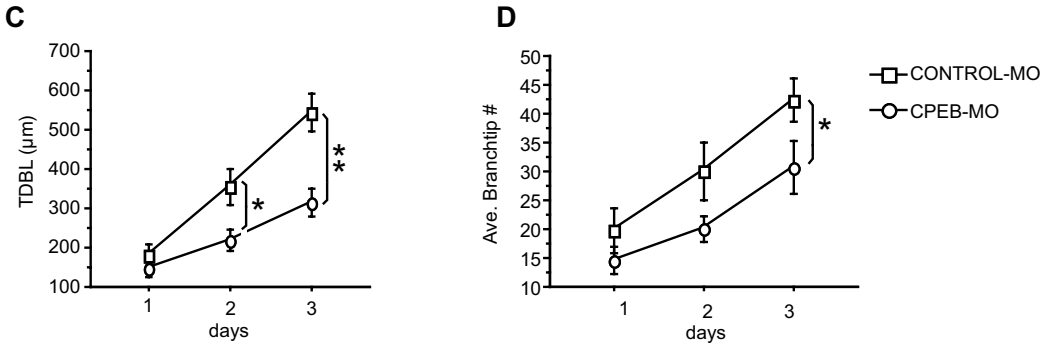
Sections were imaged with an Olympus FV300 confocal using a 60× water immersion objective (1.1 NA, Olympus) equipped with Ar-Ion 488, He-Ne 543 and 633 laser lines. A 510–530 bandpass, 585, and 660 long-pass filters were used. Image stacks of 1-μm z intervals were taken sequentially.

All analyses were conducted using Metamorph (MDS Analytical Technologies) blind to genotype of the transfected cell. After locating a transfected cell, the optical section with the highest propidium iodide fluorescence was found and, using that signal, a region of interest was drawn around the soma and then transferred to the tPA immunofluorescence channel where the average fluorescence intensity per pixel was determined. An untransfected neighboring cell to the transfected cell was selected at random and the procedure repeated. The normalized fluorescence (Fl)-intensity (Int) difference was determined according to the formula: [Average Fl Int (transfected cell)–Average Fl Int (untransfected neighbor)]/Average Fl Int (transfected cell).

Western Blot. Twenty-five brains of tadpoles electroporated with full-length CPEB or delCPEB were dissected, immediately frozen on dry ice, then homogenized in radioimmunoprecipitation assay buffer, separated by SDS/PAGE, and the proteins were transferred to nitrocellulose. The blots were blocked overnight (5% milk and 0.1% TBS with Tween) and then incubated in blocking solution with CPEB1 antibody (2 μg/ml; AbCam) overnight at 4 °C and then room temperature for 2 h. The blots were then rinsed and then incubated in goat anti-rabbit HRP (1:3000) for 1 h. Detection was by chemiluminescence (Amersham). To check for equal lane loading, blots were then stripped and the procedure repeated with an antibody to beta-actin (0.5 μg/ml, AbCam). The ratio of the intensity of the CPEB band to the actin bands were measured in each lane and these values were compared between the CPEB and delCPEB lanes.

1. Koster RW, Fraser SE (2001) Tracing transgene expression in living zebrafish embryos. *Dev Biol* 233:329–346.
2. Huang YS, Carson JH, Barbarese E, Richter JD (2003) Facilitation of dendritic mRNA transport by CPEB. *Genes Dev* 17:638–653.
3. Mendez R, Richter JD (2001) Translational control by CPEB: a means to the end. *Nat Rev Mol Cell Biol* 2:521–529.





E

Average dendrite measurements ± S. E. M. collected over 3 days for CONTROL-morpholino- and CPEB-morpholino-expressing tectal neurons. * p ≤ 0.05 and **p ≤ 0.002 (Mann-Whitney U).

TOTAL DENDRITIC BRANCH LENGTH (µm)			
	Day 1	**Day 2**	**Day 3**
CONTROL-MO	180.2 ± 28.0	353.8 ± 46.9	543.0 ± 47.5
CPEB-MO	143.8 ± 19.8	217.8 ± 27.0*	313.8 ± 36.0**
BRANCH TIP NUMBERS			
	Day 1	**Day 2**	**Day 3**
CONTROL-MO	19.7 ± 3.8	30.0 ± 5.1	42.3 ± 3.8
CPEB-MO	14.6± 2.3	20.0 ± 2.1	30.7 ± 4.5*

F

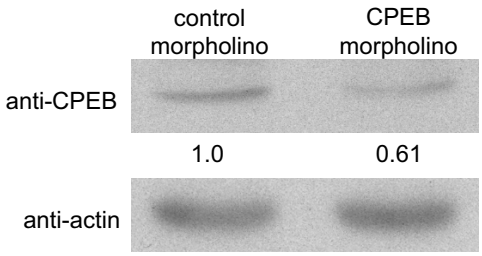


Fig. S1. Neurons expressing morpholino antisense oligonucleotides directed against CPEB fail to show experience-dependent structural plasticity. (*A* and *B*) Examples of tectal neurons expressing CPEB moropholinos (CPEB-MO) (*A*) and control 5-base mismatch morpholinos (CONTROL-MO) (*B*). Time-lapse *in vivo* two-photon images of morpholino-expressing neurons collected daily over 3 days. (*C* and *D*) Quantification of dendritic morphology of CPEB-MO-expressing neurons imaged daily over 3 days in comparison to CONTROL-MO cells. The dendritic growth of the two groups of neurons was significantly different over 3 days in total dendritic-branch length (TDBL) (*C*) or branch-tip number (*D*). (*E*) A table of the values (mean and SEMs) presented in (*C*) and (*D*). CONTROL-MO, $n = 13$; CPEB-MO, $n = 12$. (*F*) Western Blot. A CPEB antibody was used to probe the levels of CPEB protein in lysate made from CONTROL-MO and CPEB-MO electroporated brains. CPEB-MO reduces CPEB protein levels compared to CONTROL-MO.

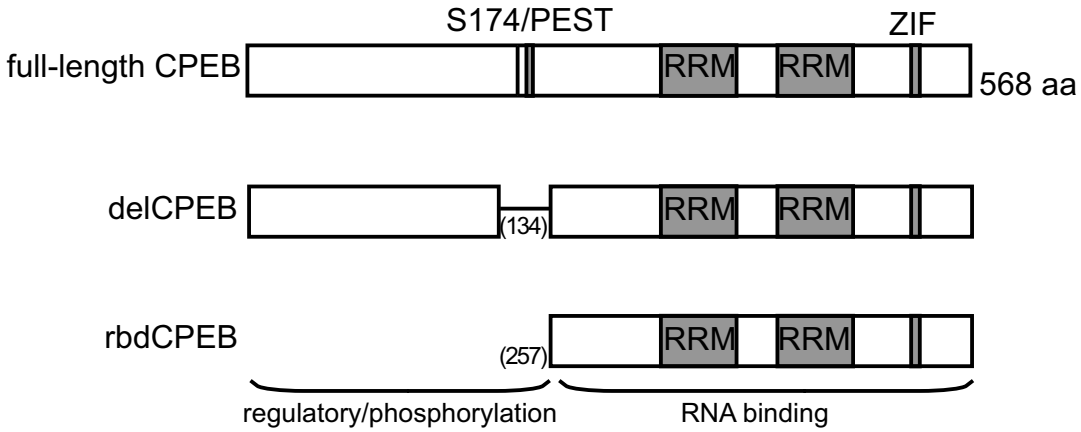


Fig. S2. Diagram of CPEB constructs. delCPEB, a CPEB deletion mutant (Δ124–258) removing the S174 that is phosphorylated to activate the protein and the PEST degradation motif; PEST, PEST degradation motif; rbdCPEB, a CPEB deletion mutant (Δ1–258) where the C-terminal portion of the protein containing the RNA binding domains is left intact; RRM, RNA recognition motif domain; ZIF, zinc finger RNA binding domain. (Modified from refs. 2 and 3.)

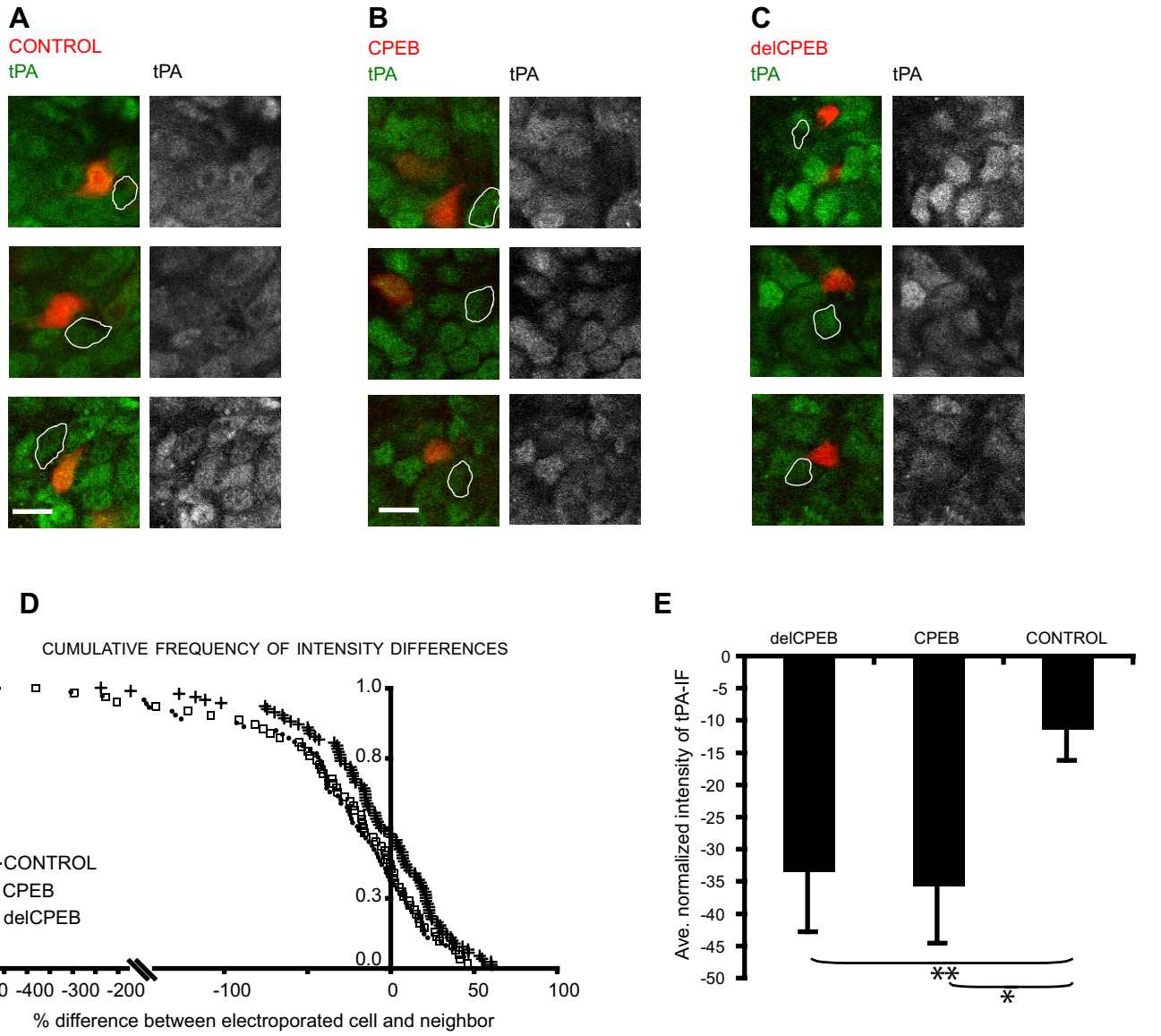


Fig. S3. Altering CPEB activity significantly decreases the amount of tPA immunofluorescence, a CPE-containing mRNA. (*A–C*) Examples of control YFP- (*A*), CPEB- (*B*), and delCPEB- (*C*) transfected cells (*Left*, *red*) and tPA immunofluorescence (*Left*, *green*; *alone*, *Right*). The tPA immunofluorescence of the transfected cells was compared to a randomly selected neighboring cell (*white outline*) and the percent differences in intensity values were calculated. Examples are arranged in increasing amounts of tPA immunofluorescence of the transfected cell compared to its neighbor. (*D* and *E*) Quantification of tPA immunofluorescence (IF). (*D*) The cumulative frequency of the normalized intensity of tPA-IF values. Both CPEB and delCPEB groups have significantly different distributions from the YFP control group. (*E*) The mean ± SEM of the normalized tPA-IF values (−11.4 ± 4.9%, control; −35.7 ± 9.1%, CPEB; −33.5 ± 9.4%, delCPEB). CPEB and delCPEB cells have significantly less tPA immunofluorescence compared to unelectroporated neighbors. Measurements were conducted blind to experimental group and were taken from 4 to 10 tissue sections made from 2 to 3 tadpoles per group; in all 92, 73, and 62 cell pairs were analyzed from the control, CPEB, and delCPEB groups, respectively. *, $P < 0.05$; **, $P < 0.02$.

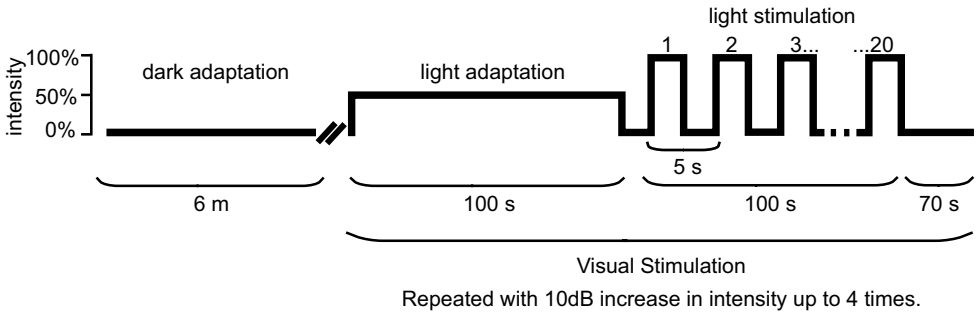


Fig. S4. Schematic of stimulation protocol. Intact, anesthetized tadpoles were dark-adapted for 6 min before the visual stimulation protocol was initiated. For each cell, the protocol was repeated up to four times, each repetition with a 10-dB (in some instances, 20 dB) increase in full field intensity. The visual stimulus protocol consisted of: 100 s light-adaptation period (the effective intensity equal to 50% of the stimulus intensity) followed by the light stimulus in which 20 repeated, full-field light flashes were presented to the animal and ending with 70 s of darkness.

Table S1. Three dimensional analysis of dendritic arbor over 3 days

	Day 1	Day 2	Day 3
Total dendritic branch length (μm)			
ControlMO	180.2 ± 28.0	353.8 ± 46.9	543.0 ± 47.5
CPEBMO	143.8 ± 19.8	217.8 ± 27.0*	313.8 ± 36.0**
Branch-tip numbers			
ControlMO	19.7 ± 3.8	30.0 ± 5.1	42.3 ± 3.8
CPEBMO	14.6 ± 2.3	20.0 ± 2.1	30.7 ± 4.5*

Average dendrite measurements ± SEM collected over 3 days for neurons expressing control and CPEB morpholinos (MO). *$P \leq 0.05$ and **$P \leq 0.005$ (Mann-Whitney U).

Table S2. Three dimensional analysis of dendritic arbor over 3 days

	Day 1	Day 2	Day 3
Total dendritic branch length (μm)			
Control	293.0 ± 45.0	620.8 ± 62.6	843.2 ± 62.4
CPEB	336.4 ± 69.0	505.5 ± 68.4	632.7 ± 69.3
delCPEB	241.6 ± 40.4	376.8 ± 59.8*	455.7 ± 78.9**
rbdCPEB	409.0 ± 55.1	581.9 ± 47.2	781.0 ± 85.8
Branch-tip numbers			
Control	20.0 ± 3.8	46.1 ± 6.1	60.1 ± 7.0
CPEB	20.9 ± 5.2	38.8 ± 6.9	43.4 ± 6.2
delCPEB	14.2 ± 2.5	27.4 ± 4.7	35.4 ± 7.9*
rbdCPEB	22.5 ± 3.2	36.0 ± 5.3	45.7 ± 5.9

Average dendrite measurements ± SEM, collected over 3 days for control-, CPEB-, delCPEB, and rbdCPEB-expressing tectal neurons. Significant difference from the control group: *$P \leq 0.05$ and **$P \leq 0.005$ (Mann-Whitney U).

Table S3. Dendritic arbor growth with and without visual stimulation

	0 h	4 h	8 h
Total dendritic branch length (μm)			
Control	478.1 ± 81.4	506.4 ± 80.0	619.4 ± 89.9**
CPEB	433.8 ± 48.7	412.9 ± 31.1	488.0 ± 28.6**
delCPEB	389.8 ± 35.0	397.2 ± 31.8	403.3 ± 33.7
rbdCPEB	509.9 ± 42.0	538.6 ± 44.9	586.4 ± 53.8
Branch-tip numbers			
Control	38.5 ± 7.5	39.9 ± 7.4	50.1 ± 8.8*
CPEB	33.0 ± 4.2	30.4 ± 4.0	42.9 ± 4.0**
delCPEB	35.5 ± 4.6	34.9 ± 4.6	35.4 ± 4.6
rbdCPEB	38.5 ± 3.6	43.5 ± 3.9*	43.7 ± 5.3

Average dendrite measurements before visual deprivation (0 h), and before (4 h) and after (8 h) the visual stimulation. Significant changes in a 4 h period: *$P \leq 0.05$, **$P \leq 0.005$ (Mann-Whitney U).

Table S4. Average growth rates of dendritic arbors during 4 h with and without visual stimulation

	TDBL (μm)		Branch tips	
	Dark	Light stimulation	Dark	Light stimulation
Control	28.3 ± 14.5	113.0 ± 21.7*	1.4 ± 3.7	10.2 ± 3.8
CPEB	−20.9 ± 24.4	75.2 ± 14.5**	−2.6 ± 2.8	12.5 ± 1.4**
delCPEB	7.5 ± 13.7	6.1 ± 10.7	−0.6 ± 2.0	0.6 ± 1.7
rbdCPEB	28.8 ± 19.7	47.4 ± 24.8	5.1 ± 6.2	0.2 ± 2.9

Changes in TDBL and branch-tip numbers over 4 hours. Significant difference from control group in the growth rates measured during the visual deprivation period and visual stimulation period: *$P \leq 0.01$, **$P \leq 0.005$ (Mann-Whitney U).

Table S5. Branching behavior and growth of individual branches during the 4-h periods with and without visual stimulation

	Dark	Light stimulation	Dark	Light stimulation
	% New branches		% Lost branches	
Control	57.5 ± 5.3	62.9 ± 4.1	51.4 ± 6.1	55.0 ± 5.0
CPEB	49.1 ± 6.2	59.3 ± 3.3	54.4 ± 5.7	39.6 ± 5.1
delCPEB	46.1 ± 4.1	45.0 ± 3.4**	47.6 ± 2.3	48.0 ± 2.6
	branch length extension (μm)		Branch length retraction (μm)	
Control	5.4 ± 0.3	6.2 ± 0.3	4.9 ± 0.2	4.8 ± 0.3
CPEB	5.0 ± 0.3	4.6 ± 0.2**	4.9 ± 0.3	4.9 ± 0.3
delCPEB	4.4 ± 0.2**	4.0 ± 0.2**	4.1 ± 0.2*	3.8 ± 0.1**

Significant difference from control group: *$P \leq 0.05$, **$P \leq 0.01$ (Mann-Whitney U).

Table S6. Average responses of tectal neurons to full field visual stimuli

Total charge transfer (pA/s)

Stimulus Intensity	0.01	0.1	1	10
Control	42.6 ± 13.1	75.0 ± 24.4	86.3 ± 20.7	106.3 ± 35.2
CPEB	24.6 ± 6.5	46.1 ± 10.8	32.6 ± 15.2*	30.0 ± 8.3**
delCPEB	8.1 ± 2.3*	9.7 ± 3.4**	6.9 ± 2.7***	4.6 ± 2.9***

Average charge transfer (Q) over the initial 500 ms of the light-off response of tectal neurons. Stimuli of increasing 10-dB intensities (10^{-3} to 10^{+1}) were presented to the intact animal. Significant difference from the control group: $*P \leq 0.05$, $**P \leq 0.01$, $***P \leq 0.005$ (Mann-Whitney U).

Table S7. Spontaneous activity of tectal neurons recorded during the dark

	EPSC amplitude (pA)	IEI (s)
Control	35.5 ± 0.7	0.12 ± 0.10
CPEB	31.7 ± 0.8*	0.57 ± 0.06*
delCPEB	28.7 ± 0.5*	1.3 ± 0.10*

Average EPSC (pA) and inter-event interval (IEI; s) during the initial 6-min dark period that preceded each visual stimulation experiment. Significant difference from the control group: *$P \leq 0.0001$ (Mann-Whitney U).