Supplemental Figures

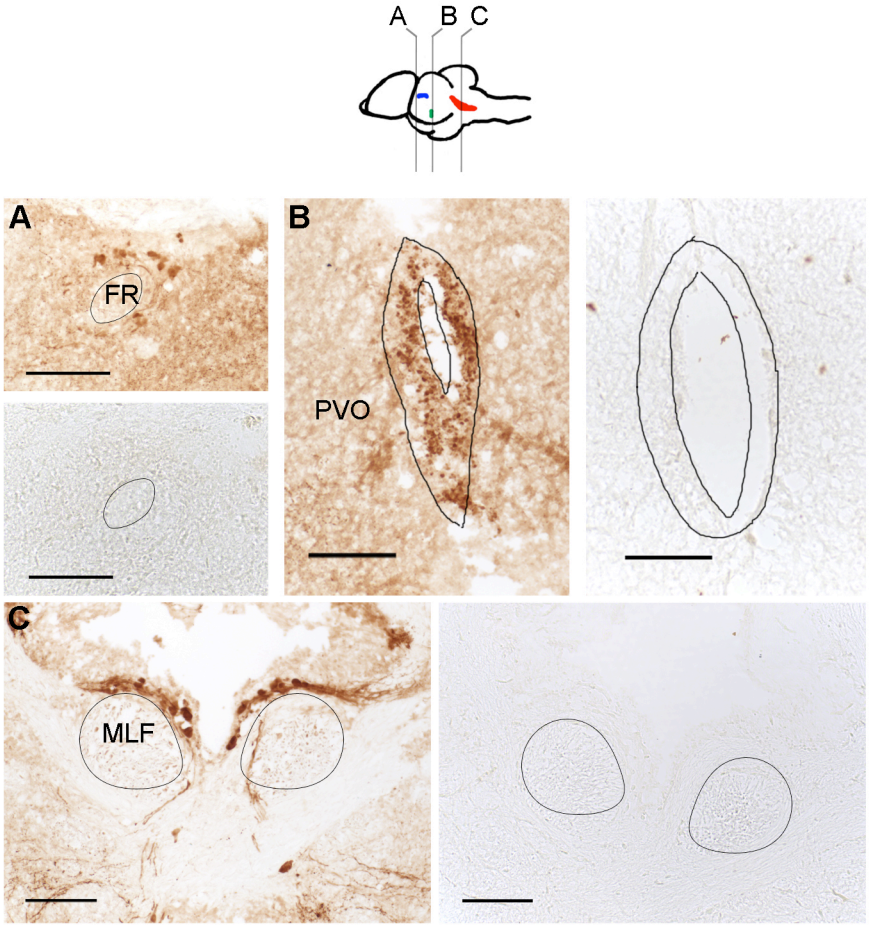

Figure S1. Preadsorption controls for 5-HT immunohistochemistry. No staining was present in immunohistochemistry controls where the primary antibody was preadsorbed with 5-HT BSA conjugate prior to incubation with tissue. For each panel (A-C) adjacent coronal sections from the same animal are shown with 5-HT staining (brown) and with preadsorbed antibody. Dorsal periventricular pretectal nucleus (A), nucleus of the paraventricular organ (PVO) (B) and raphe (C). FR, fasciculus retroflexus tract; MLF, medial longitudinal fasciculus tract. Scale bar = 100 μm.

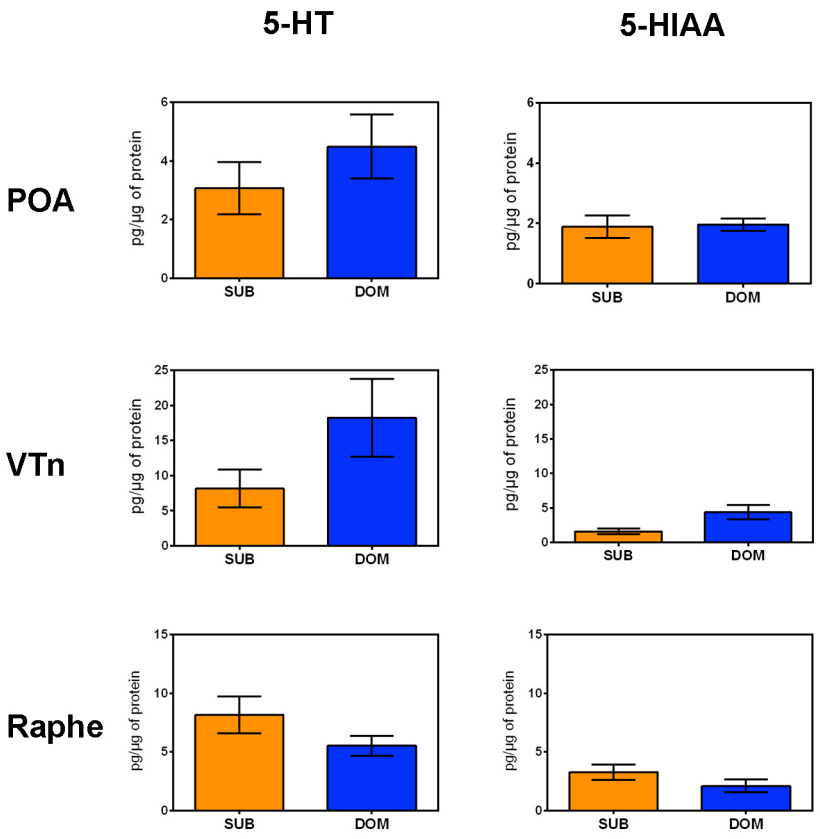

Figure S2. Levels of serotonin (5-HT) and its catabolite, 5-hydroxyidoleacetic acid (5-HIAA), in the preoptic area (POA), ventral tuberal nucleus (VTn) and raphe by social group. Levels of 5-HT and 5-HIAA were measured in microdissected tissue from the POA, VTn and raphe of subordinate (SUB, orange) and dominant (DOM, blue) males; there were no significant differences between groups. Data was normalized to the amount of protein measured in each sample, and is expressed as pg per µg of protein.

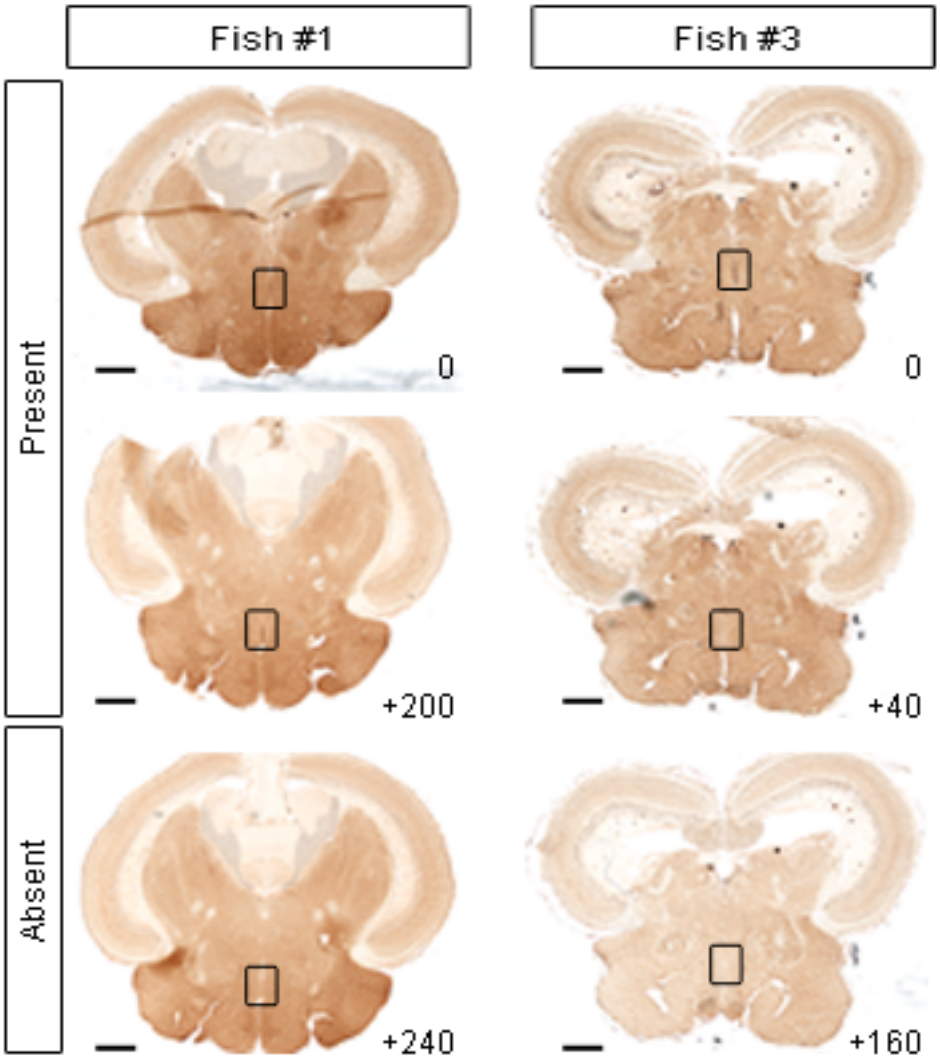

Figure S3. Representative photomicrographs of the *Astatotilapia burtoni* male brain showing 5-HT-ir cells in the nucleus of the paraventricular organ (PVO). Each column consists of three sequential coronal sections (anterior to posterior) for a given male fish. The top two rows depict sections with positively stained cells in the PVO (boxed area). In the bottom row, the same relative location of the PVO is boxed for a more posterior section where stained cells are absent. Distance (μm) from the first section is noted in the lower right corner of each section. The purpose of these images is to serve as reference for comparison to descriptions in other species (see Table S1 for details on PVO nomenclature). Scale bars = 500 μm

Table S1

Table of representative examples in three fish species for nomenclature variations of hypothalamic populations containing 5-HT-ir cells

This table lists the names reported in publications that describe the location of hypothalamic populations containing 5-HT-ir cells in zebrafish (*Danio rerio*), Mexican cavefish (*Astyanax mexicanus* and bluebanded goby (*Lythrypnus dalli)*. Each row represents a given hypothalamic population or brain nucleus and each column shows information from a single publication. The purpose of this table is to illustrate the wide range of terms used to describe these populations. The column (Rink and Wulliman, 2001) is referenced as a source to show corresponding names according to earlier versions of the zebrafish brain atlas. In this study, the 5-HT-ir staining we report for the hypothalamus belongs to the PVO (nucleus of the paraventricular organ), which to the best of our knowledge, corresponds to the descriptions listed in the first row of this table.

Species	*Danio rerio,* zebrafish			*Astyanax mexicanus,* cavefish	*Lythrypnus dalli,* bluebanded goby
Reference	**(Lillesaar, 2011)**	**(Kaslin and Panula, 2001)**	**(Rink and Wullimann, 2001)**	**(Elipot et al., 2012)**	**(Lorenzi and Grober, 2012)**
Abbreviation *Name or description*	**PT** posterior tuberculum	**PVOa** anterior part of the paraventricular organ	**PVO** paraventricular organ	**PVa** anterior part of the paraventricular organ	**NPPv** posterior periventricular* nucleus
Abbreviation *Name or description*	**PT** posterior tuberculum, located posteriorly and extend laterally	**PVOi** intermediate part of the paraventricular organ	**IN** Intermediate nucleus of hypothalamus	**PVi** intermediate part of the paraventricular organ	**NRL** nucleus of the lateral recess
Abbreviation *Name or description*	Hypothalamic	**PVOp** posterior part of the paraventricular organ;	**HC** caudal zone of periventricular* hypothalamus	**PVp** posterior part of the paraventricular organ	**NRP** nucleus of the lateral recess

* The term ‘periventricular’ is underlined to emphasize its contrast to ‘paraventricular’.

Elipot, Y., Hinaux, H., Callebert, J. and Rétaux, S. (2012). Evolutionary shift from fighting to foraging in blind cavefish through changes in the serotonin network. *Curr. Biol.***23**, 1-10.

Kaslin, J. and Panula, P. (2001). Comparative anatomy of the histaminergic and other aminergic systems in zebrafish (*Danio rerio*). *J. Comp. Neurol.* **440**, 342–377.

Lillesaar, C. (2011). The serotonergic system in fish. *J. Chem. Neuroanat.* **41**, 294–308.

Lorenzi, V. and Grober, M. S. (2012). Immunohistochemical localization of serotonin in the brain during natural sex change in the hermaphroditic goby *Lythrypnus dalli*. *Gen. Comp. Endocrinol.* **175**, 527–536.

Rink, E. and Wullimann, M. F. (2001). The teleostean (zebrafish) dopaminergic system ascending to the subpallium (striatum) is located in the basal diencephalon (posterior tuberculum). *Brain Res.* **889**, 316–330.

Table S2

Correlations between 11-ketotestosterone (11-KT) and gonadosomatic indices (GSI) and the different measurements of serotonin turnover in discrete brain nuclei

Correlations of parameters of reproductive capacity (circulating 11-KT and GSI) versus levels of 5-HT, 5-HIAA and 5-HIAA/5-HT measured in tissue microdissected from the preoptic area (POA), ventral tuberal nucleus (VTn) and raphe. *R* represents the correlation coefficient with corresponding *P*-values. Numbers in bold were statistically significant at **P*<0.05.

		5-HT		5-HIAA		5-HIAA/5-HT	
Brain region		*R*	*P-value*	*R*	*P-value*	*R*	*P-value*
POA	11-KT	0.27	0.33	0.26	0.35	-0.23	0.40
	GSI	0.15	0.60	-0.37	0.17	**-0.52**	**0.048***
VTn	11-KT	0.12	0.62	0.02	0.94	0.28	0.26
	GSI	0.34	0.16	0.27	0.27	0.07	0.80
Raphe	11-KT	-0.38	0.10	-0.36	0.14	-0.24	0.34
	GSI	-0.05	0.84	-0.19	0.46	**-0.48**	**0.04***

Table S3

Correlations by social group between gonadosomatic index (GSI) and 5-HIAA/5-HT measured in discrete brain nuclei

Correlations of GSI versus 5-HIAA/5-HT measured in tissue microdissected from the preoptic area (POA), ventral tuberal nucleus (VTn) and raphe are shown separately for each social group. *R* represents the correlation coefficient with corresponding *P*-values. Numbers in bold were statistically significant at ***P*<0.01.

Brain region	Subordinate		Dominant	
	R	*P-value*	*R*	*P-value*
POA	-0.41	0.33	-0.14	0.78
VTn	-0.38	0.36	-0.28	0.43
Raphe	**-0.83**	**0.005****	0.25	0.52

Table S4

Correlations of behaviors associated with dominant status and 5-HIAA/5-HT levels in discrete brain nuclei

Correlations between specific dominance behaviors and 5-HIAA/5-HT measured in preoptic area (POA), ventral tuberal nucleus (VTn) and raphe. For each animal, the total number of times a given behavior was displayed over the 30 min period immediately before time of sacrifice, was used as a single data point in the behavior data set. *R* represents the correlation coefficient with corresponding *P*-values. Numbers in bold were statistically significant at **P*<0.05, ***P*<0.01.

	Quiver	Lateral display	Lead	Border fight
POA				
R	0.61	**-0.86**	**0.85**	0.00
P-value	0.15	**0.02***	**0.03***	> 0.99
N	7	7	7	7
VTn				
R	**-0.85**	0.35	-0.62	-0.02
P-value	**0.003****	0.24	0.10	0.96
N	9	13	8	11
Raphe				
R	-0.13	-0.25	0.60	0.18
P-value	0.73	0.48	0.09	0.64
N	9	9	9	9

Table S5

Amplicon melting temperatures for genes used in qPCR assays

Gene	Amplicon melting temperature (^{o}C)
gapdh	77.5
rpl32	82
htr1a	82.5
htr1d	83
htr2a	79
htr3b	79.5
htr6	85

Table S6

Primer sequences and accession numbers for genes used in qPCR assays

Gene	Forward primer 5'-3'	Reverse primer 5'-3'	Accession number
gapdh	CAC ACA AGC CCA ACC CAT AGT CAT	AAC CAC ACT GCT GCT GCC TAC ATA	AF123727
rpl32	TGC TGA TGC CCA ACA TCG GTT	TCT TGG AGG AGA CAT TGT GGG	XM005931788.1
htr1a	ATG TAG CAC GAC TCC TGG CAG AAA	TCA TCA TGG GCA CCT TCA TCC TCT	GQ221844.2
htr1d	TCC ACA CAC CTG CCA ACT TCC TAA	GCG CAA TTA CAC ACA GGT GCA AGA	GQ227406
htr2a	CAT CCA TCA TGC ACC TTT GTG CCA	GAA TTG AAG CGG CTG TGG TGG ATT	GQ227409
htr3b	ACT GCT GTC CAT TCC TTC ACG CTA	GCC GGC GAA TCA ACA CGT TAA ACT	GQ227408
htr6	TCG ATC TAT GGT GGC ATT GGT GGT	GCT GCA GCA CAT CAC ATC AAA GCA	GQ227403