Lateralized Feeding Behavior is Associated with Asymmetrical Neuroanatomy and Lateralized Gene Expressions in the Brain in Scale-Eating Cichlid Fish

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Abstract

Lateralized behavior ("handedness") is unusual, but consistently found across diverse animal lineages, including humans. It is thought to reflect brain anatomical and/or functional asymmetries, but its neuro-molecular mechanisms remain largely unknown. Lake Tanganyika scale-eating cichlid fish, *Perissodus microlepis* show pronounced asymmetry in their jaw morphology as well as handedness in feeding behavior—biting scales preferentially only from one or the other side of their victims. This makes them an ideal model in which to investigate potential laterality in neuroanatomy and transcription in the brain in relation to behavioral handedness. After determining behavioral handedness in *P. microlepis* (preferred attack side), we estimated the volume of the hemispheres of brain regions and captured their gene expression profiles. Our analyses revealed that the degree of behavioral handedness is mirrored at the level of neuroanatomical asymmetry, particularly in the tectum opticum. Transcriptome analyses showed that different brain regions (tectum opticum, telencephalon, hypothalamus, and cerebellum) display distinct expression patterns, potentially reflecting their developmental interrelationships. For numerous genes in each brain region, their extent of expression differences between hemispheres was found to be correlated with the degree of behavioral lateralization. Interestingly, the tectum opticum and telencephalon showed divergent biases on the direction of up- or down-regulation of the laterality candidate genes (e.g., *grm2*) in the hemispheres, highlighting the connection of handedness with gene expression profiles and the different roles of these brain regions. Hence, handedness in predation behavior may be caused by asymmetric size of brain hemispheres and also by lateralized gene expressions in the brain.

Key words: behavioral genetics/genomics, left-right asymmetry, neural structures, *Perissodus microlepis*, tectum opticum, telencephalon.

Introduction

Behavioral handedness (or behavioral lateralization) is the tendency of an individual to favor one side of the body over the other. Humans are behaviorally lateralized: most of us are right-handed (89%) and a minority are left-handed (8%), whereas truly ambidextrous individuals are rare (3%) (Vuoksimaa et al. 2009). A number of studies provided evidence that not only humans, but also other vertebrates such

as chimpanzees, dolphins, birds, reptiles, amphibians, and fishes, but also invertebrates do exhibit lateralized behaviors (e.g., reviewed in Vallortigara et al. 2011). For example, a preferred usage of the right hand was also observed in our closest relatives, the chimpanzees, *Pan troglodytes* (Hopkins and Cantalupo 2005). Right-eye preference for swimming counter-clockwise in bottlenose dolphins, *Tursiops truncatus* has recently been reported (Clark and Kuczaj II 2016). Behavioral biases are also documented for some birds such as parrots and cockatoos that preferentially use one foot over the other to handle food and objects more effectively (Harris 1989). Even in a spider (*Scytodes globula*) a bias in leg use during prey handling has been described (Ades and Ramires 2002). Although behavioral handedness is found across a variety of distantly related animal lineages, it is not clear how widespread it is. Nevertheless, it has been suggested that handed behavior may provide ecological benefits (Vallortigara and Rogers 2005), and it has an ancient evolutionary origin (Vallortigara et al. 1999).

Behavioral lateralization has been suggested to be associated with brain asymmetry involving neuroanatomical (structural) asymmetries and/or lateralized brain functions (Rogers and Andrew 2002). The best documented case of a correlation between behavioral lateralization and brain structural asymmetries comes from humans, where the central sulcus surface on the cerebrum in the brain was found to be greater in the left hemisphere than in the right (White et al. 1994). This human structural (cortical) asymmetry has been suggested to have a molecular genetic basis (Sun et al. 2005; Johnson et al. 2009; Hibar et al. 2015). In birds such as chickens and pigeons, differential light exposure to one eye during development gives rises to a structural asymmetry in the visual pathways that is driven by lateralized behavior (Rogers and Deng 1999). The relationship between behavioral lateralization and neural structure asymmetry has also been shown in other vertebrates such as zebrafish (Concha et al. 2000) and the convict cichlid fish, *Amatitlania nigrofasciata* (e.g., in the habenular nucleus; Gutiérrez-Ibáñez et al. 2011). However, examples of such a correlation, and possible cause-effect relationship, remain few (Gutiérrez-Ibáñez et al. 2011), and the functional basis of this association is still only poorly understood (Ichijo et al. 2017).

Evidence for the functional brain asymmetries related to behavioral lateralization is relatively well documented in humans, for example, functional hemispheric specialization on language and other cognitive functions predominantly localized in the left half of the brain, whereas spatial recognition is localized in the right (Galaburda et al. 1978; Gazzaniga 2005; Sun and Walsh 2006). Indeed, most right-handed humans show a strong language specialization towards the left brain hemisphere, whereas left-handed individuals indicate less distinct patterns (Coren 1992). Several studies of other vertebrates have further demonstrated that their two brain hemispheres also have distinctive functional roles (reviewed in MacNeilage et al. 2009). Although ordinary (naturally

occurring) behaviors such as foraging behavior were shown to be retained towards the left hemisphere in vertebrate animals examined so far, including birds, fishes, toads, baboons, and whales, wariness (e.g., in the presence of predators) or memory-based individual recognition behaviors were primarily governed by the right hemisphere (MacNeilage et al. 2009). Examples for causal relationships of laterality underlying macroscopic structural differences or size, that is, "molecular genetic associations" between lateralized behavior and differences between the brain's hemispheres have so far been documented exclusively for humans (e.g., Sun et al. 2005; Johnson et al. 2009; but see deCarvalho et al. 2014). Future study of other vertebrate species will help to advance our understanding of the neuro-molecular basis of lateralized behavior more generally.

A well-documented case of behavioral lateralization in fish is scale-eating (lepidophagous) in cichlids of the species, *Perissodus microlepis* that are endemic to Lake Tanganyika in Africa (Hori 1993; Futuyma 2009). Some individuals of this species preferentially attack the left flanks of their prey fish to bite off scales ("right-handed"), others attack the right flanks ("left-handed"), and a small minority attack both flanks with similar frequencies (no "handedness") (Lee et al. 2012). This handed behavior is already expressed early in juvenile *P. microlepis* at an age of 2 months (Lee et al. 2012), and is known to be correlated with morphological mouth asymmetry (fig. 1*A* and *B*; Hori 1993; Lee et al. 2010; Van Dooren et al. 2010; Kusche et al. 2012; Takeuchi et al. 2012, 2016), which is found to be under polygenic control (Lee et al. 2015; Raffini et al. 2016). This predicted relationship between morphological and behavioral laterality is sometimes less strong for laboratory-raised fish without prior experience feeding on scales from prey fish (Lee et al. 2012). The molecular basis or neuro-molecular mechanisms underlying this remarkable behavioral laterality remain unknown and are the focus of this study.

Genome-wide RNA sequencing (RNA-Seq) permits the exploration of the functional basis of the expressed genes relatively quickly and effectively (Conesa et al. 2016). In particular, RNA-Seq facilitates the study of the gene regulatory networks of ecologically or evolutionarily intriguing traits in nonmodel organisms, even if their genomes have not been sequenced yet (e.g., Elmer et al. 2010). Several recent studies using RNA-Seq have been performed to study gene expression patterns and genetic pathways underlying key ecological traits in fishes (e.g., Gunter et al. 2013; Henning et al. 2013; Manousaki et al. 2013; Kang et al. 2015). Yet, the genetic underpinnings of adaptive behavioral phenotypes and the role of gene expression differences in regulating behavior remain largely unidentified (e.g., Whitfield et al. 2003; Aubin-Horth et al. 2005; Renn et al. 2008; Drew et al. 2012; Harris and Hofmann 2014).

In the present study, we investigated whether lateralized foraging behavior in the Lake Tanganyika scale-eating cichlid fish, *Perissodus microlepis*, is correlated with brain neuroanatomical (structural) asymmetry (fig. 1*C* and *D*). We also

performed transcriptome analyses on different regions of the fish's brains and on both left and right hemispheres in order to link behavioral handedness to gene transcriptional differences between hemispheres in *P. microlepis*. For this, we investigated whether genes showed a correlation between the degree of gene expression fold-change between hemispheres and that of lateralized behavior. The specific objectives of this study were 4-fold: 1) to test whether behavioral handedness in *P. microlepis* is related to left-right asymmetry in neural structures; 2) to explore the tissue-specific gene expression profiles regardless of an individuals' handed feeding behaviors and hemispheric regions; 3) to investigate if the differences in gene expression in each paired bilaterally symmetrical brain region (i.e., tectum opticum, telencephalon, and hypothalamus; fig. 1D) between left and right hemispheres can be linked to the degree of lateralized behavior; and 4) to identify potential candidate genes in each brain region that might underlie behavioral laterality. The results of our study will open a novel perspective on the functional genomic basis of this remarkable case of behavioral laterality and lay the ground for future research on behavioral genetics/genomics.

Materials and Methods

Samples

Twenty-seven *Perissodus microlepis* individuals were used to determine whether brain anatomical (or structural) asymmetry is correlated with behavioral foraging lateral preference. Fish were 6–10 months of age, from two laboratory-bred broods from wild-caught parents (NH × NH [no "handedness" observable, i.e. fish were showing an equal preference and attacked both flanks of prey], brood 6, $N = 22$; LH ["left-handed"]×NH, brood 9, $N = 5$) (supplementary table S1, Supplementary Material online). Twelve additional *P. microlepis* individuals (not included in the 27 fish) were used to carry out transcriptome analyses on the brain to investigate the architecture of gene regulatory mechanisms of behavioral lateralization (supplementary table S2, Supplementary Material online). These were also laboratory-bred fish, 6 months of age, from a single brood from wild-caught parents (RH ["right-handed"]×NH). Note that in additional 12 fish from brood 6 (NH × NH), morphological mouth asymmetry estimated from jaw-bending orientation in angle (°), after the heads of these fish were cleared and double-stained as done in Lee et al. (2015), was not associated with behavioral lateralization ($Y = -0.027X + 0.207$, $R^2 = 0.005$, $P = 0.836$) (Lein 2012).

The parental fish for the test batches of *P. microlepis* were obtained in April 2010 by diving with hand nets at Toby Veal's Lodge (S08°37.4' E031°12') near Mpulungu, Zambia on the southern tip of Lake Tanganyika (Kusche et al. 2012; Lee et al. 2012, 2015, 2016). The fish were kept at the animal research facility of the University of Konstanz, Germany. All the broods were reared in 40 *L* and later 200 *L* aquaria with *Artemia* nauplii

and flake food as diet under a 12 h:12 h light:dark cycle. Animal care of the fish, foraging experiments, brain dissection, brain volume measurements and subsequent molecular experiments in the laboratory were approved by the regional board of animal welfare in Germany (Regierungspräsidium Freiburg, Abteilung Landwirtschaft, Ländlicher Raum, Veterinär und Lebensmittelwesen) (permit number: 35/9185.81/G-10/96).

Foraging Preference Experiments

To determine behavioral laterality (foraging preference = handedness) in the 39 juvenile *P. microlepis*, we conducted foraging experiments in a 40 *L* experimental tank (see Lee et al. 2012). The scale-eaters were placed individually with a single prey fish (goldfish, *Carassius auratus*). The test fish was placed into the trial tank at least 4 h prior to the experiment. For each fish, foraging behavior was monitored by counting the number of attacks to the left and/or right flanks on a single goldfish until a total of 20 attacks per individual were reached (with some exceptions; supplementary tables S1 and S2, Supplementary Material online) (Lee et al. 2012). Standard length (SL) of the test fish, SL of the prey fish, average time taken per attack, and our determined behavioral preferences for the 12 fish used for brain transcriptomics are shown in supplementary table S2, Supplementary Material online. There was some variation in the length of the foraging trials (total number of attacks: $N = 17$–21) among the 12 test fish (6.7–45.0 min; mean = 25.5 ± 12 [SD] min), which might potentially affect gene expression patterns because the time since the first attack and the collection of the brain tissue varied. The number of left and right attacks, behavioral preferences and estimated brain hemisphere volumes (see below) for the 27 fish used to examine structural asymmetry are provided in supplementary table S1, Supplementary Material online.

To determine behavioral laterality (handedness), the foraging laterality index (FLI) [$\text{FLI}_{foraging} = $ (number of attacks $_{right}$ − number of attacks $_{left}$)/(number of attacks $_{right}$ + number of attacks $_{left}$)] was calculated individually (Facchin et al. 1999). Negative values of FLI indicate more frequent attacks on the prey's left flank (i.e., *P. microlepis* showed right-handed behavior), whereas positive values indicate more frequent attacks of the right flank of the prey fish (left-handed behavior; fig. 1B). For the statistical analyses, behavioral laterality was treated as a continuous variable. We also classified these fish based on the FLI into three behavioral groups, right-handed (RH) individuals, left-handed (LH) individuals, as well as individuals with no apparent handedness (NH). Fish ID 50 and 51 were considered LH fish, although binomial tests indicated marginal *P* values: 0.058 and 0.072 for fish ID 50 and 51, respectively (supplementary table S2, Supplementary Material online). More fish were tested for behavioral laterality, but we selected only 12 fish for the transcriptomic analysis (see below).

Brain Volume Measurements

To investigate the relationship between anatomical brain asymmetry and foraging behavior in *Perissodus microlepis*, brains of the 27 juvenile fish were dissected immediately after the foraging experiments and volumes of three bilaterally symmetrical brain regions (i.e., telencephalon, tectum opticum, and hypothalamus) were measured individually by the same person (E.L.) (fig. 1D), following the procedures of Pollen et al. (2007) and Gonzalez-Voyer and Kolm (2010). The test fish were euthanized by immersion in ice-slush water (2–4 °C) (Matthews and Varga 2012) right after the foraging trials and the brain volume was estimated from blind measurements with respect to the foraging type to avoid a possible observer's bias. Olfactory bulbs were excluded from this analysis because of a relatively lower repeatability ($r < 0.75$) of our volume measurements detected (see below), probably due to its relatively small size (fig. 1C and D; supplementary fig. S1, Supplementary Material online). Brains were digitally photographed using a Zeiss Axiophot digital microscope (Zeiss, Germany), and width (W), length (L), and height (H) of each of the brain structures were then measured using ImageJ 1.45r (http://imagej.nih.gov/ij; last accessed October 23, 2017) to estimate their volumes (supplementary fig. S1, Supplementary Material online for measurement illustrations). According to Pollen et al. (2007) and Gonzalez-Voyer and Kolm (2010), the width (W) was measured as the greatest distance of a particular brain region that is perpendicular to the anatomical midline, the length (L) was measured as the greatest distance by that particular brain region parallel along the axis of the brain, and height (H) was measured as the greatest distance perpendicular to the body axis for that particular brain structure (supplementary fig. S1, Supplementary Material online).

The brain volume (V) was then calculated according to an ellipsoid model (Van Staaden et al. 1995): V (in mm³)$= (L \times W \times H)\ \pi/6$. The method employed in this study is a well-established and standardized protocol for the estimation of brain volumes in fishes (e.g., Pollen et al. 2007; Gonzalez-Voyer and Kolm 2010). A Spearman rank correlation analysis was conducted to test for a significant relationship between the ratio of brain hemisphere volumes (left to right) and behavioral laterality (i.e., FLI). We used a nonparametric test for this analysis because the assumption of normality was not satisfied (FLI: Shapiro–Wilk test, $W = 0.897$, $P = 0.011$). Three different Spearman rank correlation analyses were also performed for the paired brain structures.

To assess the accuracy of these measurements, repeatability (r) of the brain volume measures was estimated from independently repeated and "blind" measurements that were undertaken from two replicate photographs of each structure of the same individuals from a subsample ($N = 13$) using one-way ANOVA with individual as the factor, as suggested in Sokal and Rohlf (1995).
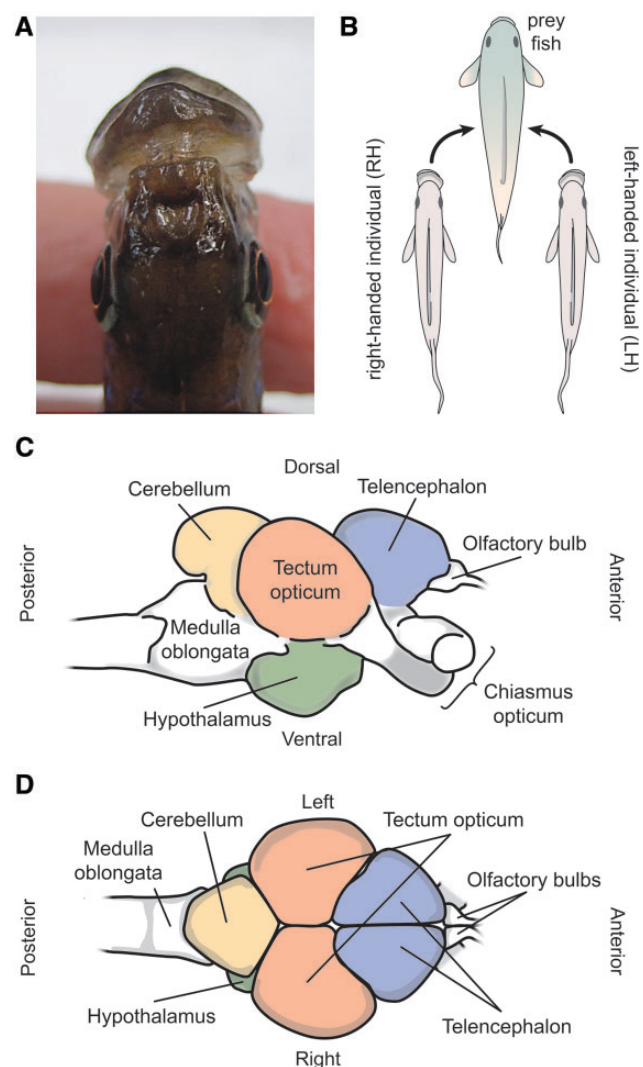

FIG. 1.—A textbook example of a correlation between mouth asymmetry and lateralized foraging behavior in the scale-eating cichlid fish, *Perissodus microlepis*, from Lake Tanganyika. Note that this expected relationship is sometimes less strong in laboratory-reared fish (Lee et al. 2012; in the current study). (A) Dorsal view of left-bending mouth morph of this species. (B) Right-bending morphs preferentially attack left flanks of the prey fish (right-handed [RH]), and left-bending morphs prefer to feed from right flanks (left-handed [LH]). (C) Lateral and (D) Dorsal illustration of a *Perissodus microlepis* brain. Brains were dissected and the paired structures, hypothalamus, tectum opticum, and telencephalon, and the unpaired one, cerebellum, were used for gene expression analyses.

Brain Transcriptome Analyses

Brain Dissection and RNA Extraction

Brains of the experimental *P. microlepis* fish ($N = 12$) were isolated using a fine scissor and forceps in 0.1 M phosphate-buffered saline (PBS) solution on ice, immediately after the end of the foraging trials. Seven different brain regions (telencephalon-L and -R, tectum opticum-L and -R, hypothalamus-L

and -R, and cerebellum) were dissected for each fish and preserved in RNAlater (Qiagen GmbH, Hilden, Germany) until RNA extraction. The tectum opticum, telencephalon, and hypothalamus are bilaterally paired symmetrical structures, whereas the cerebellum is unpaired (see fig. 1C and D; supplementary fig. S1, Supplementary Material online).

A total of 84 RNA samples from 12 fish [7 brain tissues × 4 individual fish × 3 (our determined) behavioral groups (RH, LH, and NH)] were obtained. Total RNA of each brain tissue was isolated with Trizol (Invitrogen, USA). Tissues were homogenized using pestles and mRNA was extracted with chloroform. RNA was further purified using RNeasy columns (Qiagen). On-column DNase treatment was performed following the manufacturer's protocols. Additional washing and drying steps (washed columns twice with 80% EtOH to remove all traces of salt and ethanol) followed, and samples were spun dry for 5 min. RNA was eluted in RNase and DNase free water. RNA purity was assessed by a Nanodrop (Thermo Scientific, USA) by measuring its absorbance at 260 and 280 nm and RNA integrity (RIN) was measured using a Bioanalyzer 2100 (Agilent, USA). RNA samples with 260/280 ratio > 2.0 and RIN value > 8.5 were used for downstream library preparation.

Library Construction and Sequencing

Total RNAs recovered from the 81 brain tissues of *P. microlepis* (except for three RNA samples that failed qualification criteria for RNA purity or integrity: fish ID 55 hypothalamus-L and cerebellum; fish ID 57 tectum opticum-R) were subjected to a RNA-Seq protocol. Sequencing libraries were generated using the Illumina TruSeq RNA sample preparation kit (Low-Throughput protocol) according to the manufacturer's instructions (Illumina, San Diego, CA, USA). Briefly, 500 ng of RNA was subjected to mRNA selection using poly-T oligo-attached magnetic beads followed by chemical fragmentation (5 min, 94 °C). The cleaved RNA fragments were then copied into first strand cDNA using SuperScript II reverse transcriptase (Invitrogen, USA) and Illumina proprietary random hexamer primers. After second strand synthesis using Illumina-supplied consumables, the cDNA was amplified with reagents of the same kit according to the manufacturer's protocol and ligated to barcoded adapters. The final libraries were amplified using 15 PCR cycles. Quality assessment of the libraries was performed on a Bioanalyzer 2100 (Agilent) and the quantification was carried out in the Qubit 2.0 fluorometer (Life Technologies, USA). The 81 barcoded samples were equimolar-pooled and the same pool was loaded in 7 lanes of an Illumina flowcell in order to obtain technical replication and considerable sequencing depth. Paired-end sequencing of clustered template DNA was performed on an Illumina HiSeq2000 at the Tufts University Genomics Centre in Boston (TUCF Genomics) using four-color DNA Sequencing-By-Synthesis (SBS) technology with 210 cycles (101 cycles for each paired-read and eight cycles for the barcode sequences).

Read Quality Control

After sequencing, we obtained 1,380,519,871 raw reads of 101 bp each (average reads per sample = 17,043,455) that were quality-controlled before assembly, read mapping and downstream analyses. Reads were quality-controlled (reads with a quality score <20 were discarded) and the remaining adapters were removed with the fastx toolkit v0.013 using default parameters (http://hannonlab.cshl.edu/fastx_toolkit/; last accessed October 23, 2017). Finally, only filtered reads that were >50 nucleotides in length were used for further analyses.

Transcriptome Assembly and Annotation

For the assembly, we pooled all samples and then used a subset of the filtered reads. We chose those that were at least 95 nucleotides in length and were paired after the quality control (316,268,802 reads). 114,644,340 of those were merged and collapsed to 43,142,178 longer reads. Merging was conducted with SeqPrep (https://github.com/jstjohn/SeqPrep; last accessed October 23, 2017) and collapsing with fastx toolkit. For the assembly we used 100,812,231 nonmerged pairs of reads (total 201,624,462 reads) and 57,322,170 merged pairs of longer reads (114,644,340) collapsed to 43,142,178 reads.

The assembly process was implemented in Trinity v2.4.0 (Grabherr et al. 2011) and resulted in 305,646 transcripts. To further process the assembled transcripts and to exclude spurious ones, we mapped the reads to the assembled transcriptome using Bowtie v.1.0.0 (Langmead et al. 2009) through RSEM v1.24 (Li and Dewey 2011) to eliminate those transcripts with <1% of the reads assigned to the corresponding genes (as suggested in Haas et al. 2013). We further restricted transcripts <300 nucleotides in length to a compact and meaningful transcriptome of 176,911 transcripts clustered in 147,469 loci or components. Those sequences were used in a BLASTx search against the NCBI protein database *nr* (e-value threshold 1e−10). 70,004 transcripts and 48,968 loci had a significant hit in *nr*. Those that did not have a hit were afterwards used in a BLASTn search against NCBI nucleotide database *nt* (e-value threshold 1e−10). Finally, after retaining the sequences that had a significant hit in *nr* and/or *nt* we obtained 83,715 loci encompassing 110,331 transcripts that constituted our final assembly (N50: 798 bp).

Mapping and Expression Profiling

For mapping, we employed all high quality reads (1,023,729,409 reads, paired reads: 638,495,280 and single: 385,234,129) (supplementary table S3, Supplementary Material online). Mapping was conducted with Bowtie with RSEM software as implemented in the script *run_RSEM_align_n_estimate.pl* provided by Trinity. 722,315,809 reads (∼71%) were mapped to the assembly

and assigned to the different isoforms. Expression values were estimated using RSEM at both transcript and gene levels. Given the lack of confidence in defining isoform in de novo assemblies, we conducted the expression profiling at the gene level. Paired and "orphaned" (i.e., only one of the two reads survived the filtering process) reads were mapped separately and then pooled, which provided the final count table for all 83,715 loci. The sequences of these loci were remapped to the Nile tilapia (*Oreochromis niloticus*) genome to retrieve protein IDs and to discard unmapped, ambiguous loci. 63,389 loci were successfully annotated with 19,145 different protein IDs and loci with the same protein IDs were summed up subsequently. The resulting count table of 19,145 proteins was normalized per sample by dividing each protein ID's count by the median of all counts of that sample (supplementary table S4, Supplementary Material online). This normalization approach was chosen as pronounced differential expression between tissues was expected and other normalization approaches, such as used in DESeq2, rely on the assumption that only relatively few genes are differentially expressed (Dillies et al. 2013). As gene length was not considered for this normalization, reliable comparisons of expression levels among different genes were not feasible. Finally, gene names and gene ontology (GO) terms were retrieved for each protein ID from Ensembl. In spite of this, we will refer to these IDs with their corresponding gene names throughout the paper as gene expression was actually measured.

Analyses of Gene Expression Profiles

The transcriptional profiles of all 81 samples were investigated using a principle component analysis (PCA) to illustrate overall divergence in transcriptional patterns of the brain regions. Next, genes differentially expressed between brain regions were identified using pair-wise comparisons ("fdr"-corrected *P* values of Welch ANOVAs). Finally, gene ontology (GO) term composition of the differentially expressed genes ("test set") was compared with the composition of all 19,145 genes found in all brain regions pooled ("reference set") using Blast2GO (Conesa et al. 2005).

Differences in gene expression between the left and right hemispheres in the three paired brain regions were explored based on the PCA. Also, differentially expressed genes between the two hemispheres per brain region were determined as was done for the brain regions. To identify genes potentially underlying behavioral laterality, for each individual, paired brain region and gene, expression fold change was calculated by dividing the normalized gene expression value of the behaviorally preferred prey flank (e.g., in a fish with negative FLI preferentially attacked the left flank of the prey) by the value of the other nonpreferred side. The log2 of these values was then calculated, that is, if in a RH-fish a gene was upregulated in the left brain hemisphere, the log2 fold-change was a positive value and if it was upregulated in the

right hemisphere the log2 fold-change was a negative value. To link the degree of behavioral lateralization to the fold change in gene expression, linear regression models were calculated for each brain region and each gene, using the absolute value of FLI (i.e., values from 0.1 to 0.8) as an independent variable and log2 fold-change of the gene as a dependent variable. Candidate genes were chosen by selecting models that showed a significant slope ($P < 0.05$). Additionally, genes with a model intercept potentially different from 0 ($P < 0.1$) were discarded as these would not fulfill the assumption that behaviorally no handed individuals have no differences in gene expression between the hemispheres. No further posthoc corrections were applied after these selection steps as our results suggested that obtained overall patterns are highly unlikely coincidental and remaining candidate genes are thus likely to be mostly "true positives" (see Results section). Nonetheless, the chosen approach is more liberal than some analyses like DESeq2 and thus particularly less significant genes should be interpreted with caution. GO term composition of these brain region-specific candidate genes was explored. Results of GO term analyses were reported and discussed only for "biological processes", and those for "molecular function" and "cellular component" were also summarized. GO analyses were conducted using Blast2GO. All analyses of gene expression were conducted in R 3.3.0 (R Core Team 2016).

Results

Relationship between Brain Anatomical Asymmetry and Handed Feeding Behavior

Our measurements of brain hemisphere volumes were highly repeatable—estimated repeatability (r) was 0.88 (left [L] side) and 0.89 (right [R] side) for the telencephalon, 0.78 (L) and 0.88 (R) for the tectum opticum, and 0.91 (L) and 0.87 (R) for the hypothalamus. These estimates of the repeatability suggest that $78 - 91\%$ of the total observed variation results from underlying "true" variation in the brain hemisphere volumes among individuals, and the remaining $9 - 22\%$ variation is caused by measurement error.

A significant negative correlation was found between the ratio of the brain hemisphere volumes and the lateralized foraging behavior [Spearmans' rho (ρ) $= -0.394$, $N = 27$, $P = 0.042$; fig. 2A), which suggests that left-handed (LH) fish tend to have a larger right hemisphere than left hemisphere, whereas right-handed (RH) fish have a relatively larger left hemisphere (fig. 2A). At the brain-structure level only the tectum opticum contributed to this observed significant negative relationship ($\rho = -0.455$, $N = 27$, $P = 0.017$; fig. 2B). The other brain regions appear to be symmetrical (telencephalon: $\rho = -0.302$, $N = 27$, $P = 0.125$; fig. 2C, hypothalamus: $\rho = -0.186$, $N = 27$, $P = 0.353$; fig. 2D), albeit a similar trend.
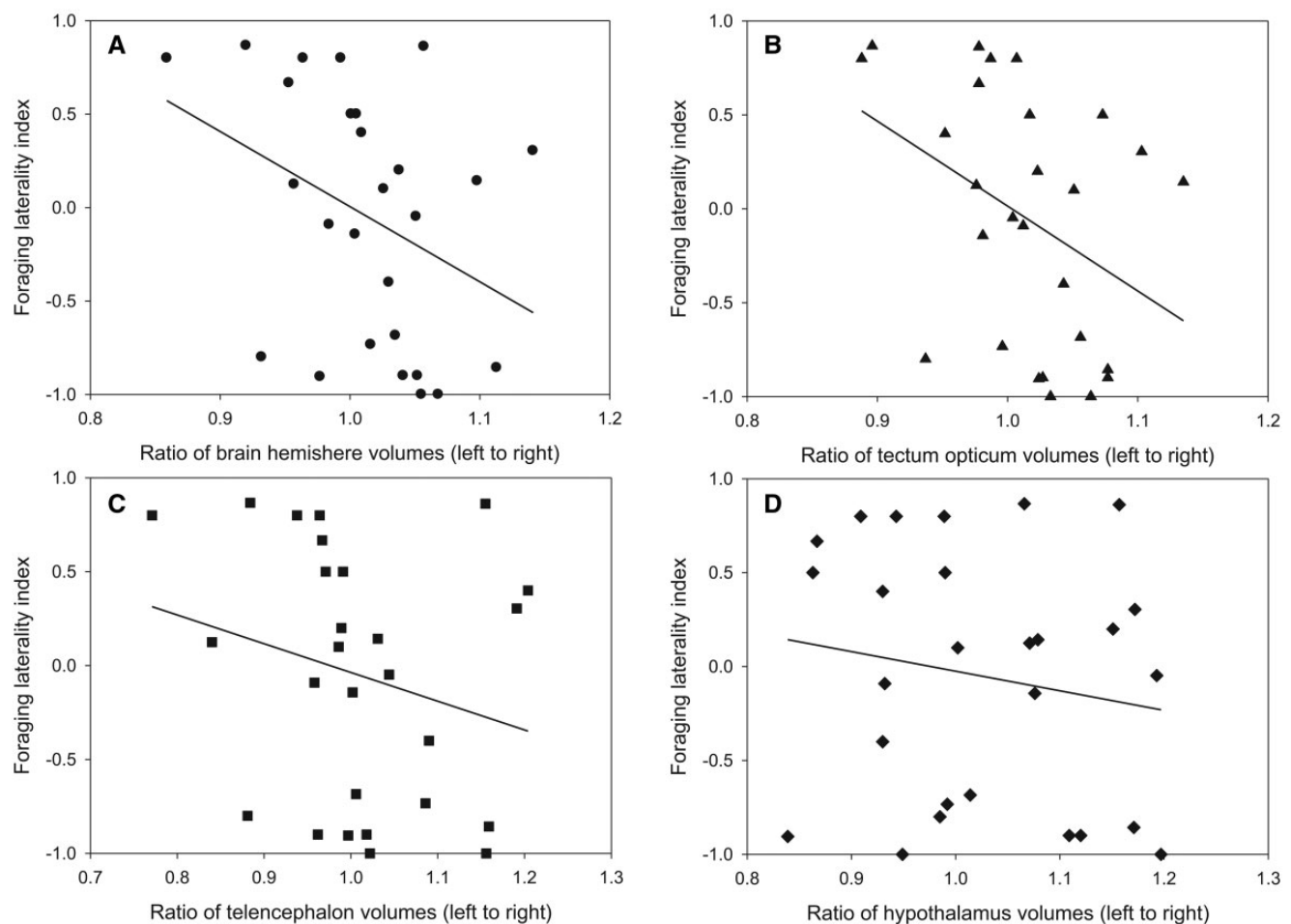
FIG. 2.—Relationship between brain anatomical left–right asymmetry and lateralized foraging behavior in *P. microlepis*. (*A*) A significant negative correlation was detected between ratio of brain hemisphere volumes (left to right) and foraging laterality index [Spearmans' rho (ρ) = −0.394, $N = 27$, $P = 0.042$]. The brain structures used for the estimates of brain hemisphere volume include tectum opticum (*B*), telencephalon (*C*), and hypothalamus (*D*). At the brain-structure level, only the tectum opticum showed a significant negative relationship ($\rho = -0.455$, $N = 27$, $P = 0.017$).

Global Expression Patterns

To estimate the relative abundance of transcripts for each locus, we mapped the 1,023,729,409 high quality reads of the 81 samples to the assembly and assessed their expression profiles at the gene level. Global expression patterns of the 81 samples are shown in a PCA plot (fig. 3; supplementary fig. S2, Supplementary Material online). Principle components (PCs) 1–3, explaining together ~86% of the total variation, showed pronounced differences among the four brain regions investigated (fig. 3; supplementary fig. S2, Supplementary Material online). The cerebellum's gene expression profile appeared to be most divergent from the other three brain regions, as PC1 suggests. Although the telencephalon and hypothalamus load similarly on PCs 1 and 2, PC3 showed a clear distinction between these two regions (supplementary fig. S2, Supplementary Material online). None of the PCs accounting cumulatively for ~95% of total variation

showed any differences between the left and right hemispheres for any brain region (supplementary fig. S2, Supplementary Material online).

A large number of genes showed differential expression in pair-wise comparisons of brain regions (left and right hemispheres pooled) after fdr corrections (table 1). Notably, approximately three quarters of the 19,145 genes expressed in the brain were differentially expressed between the cerebellum and any other brain regions, when each of the genes was tested. Comparing the hypothalamus to the telencephalon and to the tectum opticum resulted in 7,911 and 7,264 differentially expressed genes, respectively, and 11,370 genes were found to be differentially expressed between the tectum opticum and the telencephalon. In the GO term analyses we found that in the pair-wise comparisons of tectum opticum, telencephalon, and hypothalamus no highly represented GO category (in % sequences) appeared to be over- or underrepresented in the differentially expressed genes compared
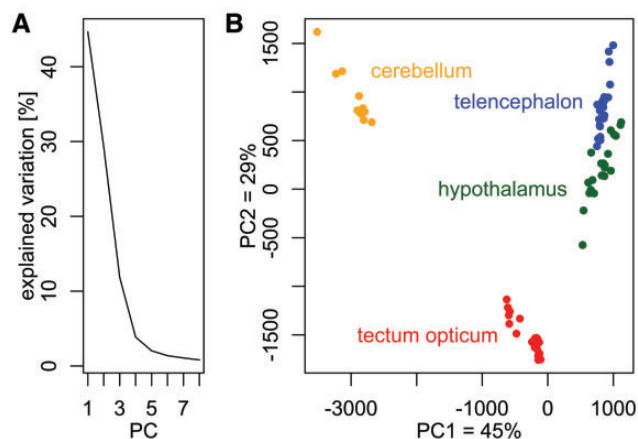
FIG. 3.—PCA (Principal Component Analysis) of normalized count data from all 81 RNA samples. (A) A scree plot showing the percentage of explained variation per principal component (PC). The first eight PCs explained ~95% of all variation of the dataset. (B) Scatter-plot of PC1 and PC2 (~74% of variation). Gene expression profiles were distinct among the four brain regions, particularly the cerebellum, and the tectum opticum was different from the telencephalon and the hypothalamus, which had somewhat more similar loadings on PC1 and PC2.

Table 1

Number of Differentially Expressed Genes between Different Brain Regions Irrespective of an Individual's Attack Behaviors and Brain Region Hemisphere

Brain regions	Telencephalon	Hypothalamus	Cerebellum
Tectum opticum	11,370	7,911	14,765
Telencephalon	—	7,264	15,133
Hypothalamus		—	14,874

with the overall reference gene-set (supplementary fig. S3, Supplementary Material online). Instead, most analyzed genes were represented in many GO categories (included in the "others" category). In contrast, when the tectum opticum and the hypothalamus were individually compared with the cerebellum, large percentages of the sequences were assigned to three and two GO categories, respectively, with different abundances between the candidate genes and the reference (supplementary fig. S3, Supplementary Material online). In both comparisons, genes associated with the GO category "integral component of membrane" were over-represented in the list of differentially expressed genes, whereas the category "nucleus" was under-represented, compared with the reference (supplementary fig. S3, Supplementary Material online). Finally, a very large proportion (~98%) of the GO terms assigned to the differentially expressed genes between the telencephalon and the cerebellum were represented by the 30 illustrated categories (supplementary fig. S3, Supplementary Material online).

We tested for differential gene expression between the left and right hemispheres in the three paired brain regions.

Welch-ANOVA was performed if at least seven nonzero expression values were available for each hemisphere. Prior correction for multiple testing, we identified 367, 370, and 469 differentially expressed genes in the tectum opticum, telencephalon, and hypothalamus, respectively. However, after false discovery rate correction, none remained significant (all P fdr > 0.05).

Gene Expression Patterns Reflecting Behavioral Laterality

For each gene and brain region, a linear model with FLI as an independent variable and gene expression as a dependent variable was calculated (linear model slope estimates irrespective of significance are shown in fig. 4A–C). For the three paired brain regions examined, including tectum opticum, telencephalon, and hypothalamus, we identified 140, 173, and 79 candidate genes, respectively, that showed a significant linear relationship with the FLI and an intercept not different from zero (table 2; supplementary table S4, Supplementary Material online). Brain regions appeared to have different biases on the direction of candidate gene slopes, that is, whereas tectum opticum-specific candidate genes mostly had a negative slope (i.e., are up-regulated in the brain hemisphere facing the prey fish), candidate genes of the other two brain regions showed the opposite trend (figs. 4D–F and 5). This was particularly pronounced in the telencephalon, where ~85% of candidate genes had a positive slope, that is, the genes were relatively up-regulated in the brain hemisphere that did not face the side at which prey fish were preferred (figs. 4E and 5). This unequal distribution of slope estimates suggests that identified candidate genes are mostly not false positives, which is why no post hoc correction was applied.

GO term analyses for biological processes suggest that laterality candidate genes in each brain region show a different composition of GO categories (fig. 6; GO term analyses for "molecular function" and "cellular component"; see supplementary fig. S4, Supplementary Material online). Notably, the laterality candidate genes of the hypothalamus were assigned to a more diverse set of GO categories ($N = 26$) than those of the tectum opticum (13) or the telencephalon (13) (this trend was also found if equal numbers of candidate genes per brain region were analyzed).

Discussion

The laterality (handedness) of the foraging behavior associated with an asymmetry in head morphology has made the scale-eating cichlid fish, *Perissodus microlepis*, from Lake Tanganyika a textbook case of the extraordinary degree of ecological adaptations (or trophic specialization) as well as for negative frequency-dependent selection (Hori 1993; Alcock 2009; Futuyma 2009) (see fig. 1A and B). However, the molecular basis or neuro-molecular mechanisms of the pronounced behavioral laterality in *P. microlepis* remained
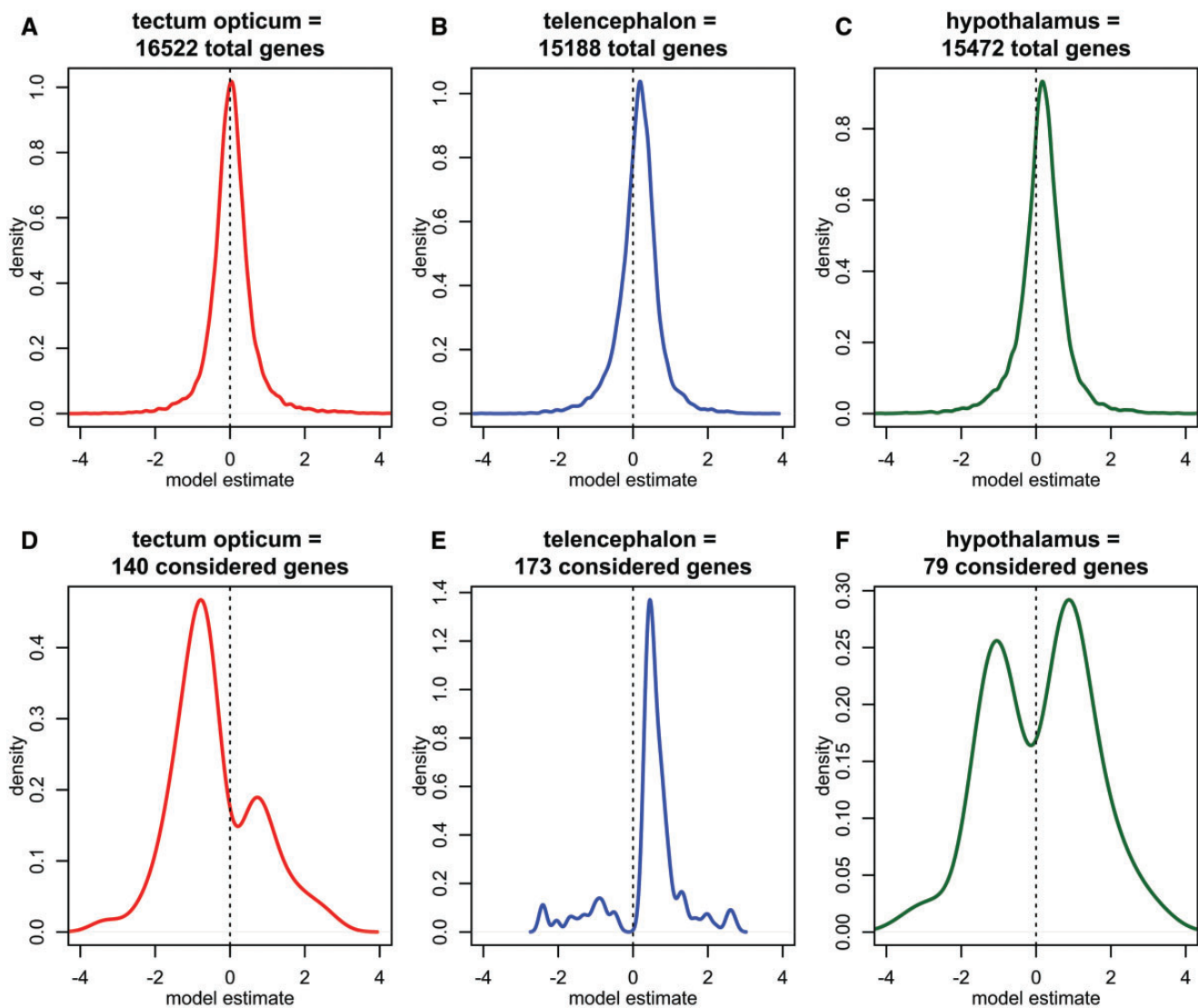
Fig. 4.—Density plots of linear model slopes for the three paired brain regions. (A–C) Density plots of the linear regression model slopes (i.e., the slope estimate) of all genes tested in the respective brain region prior to candidate gene selection. (D–F) Density plots of the linear regression model slopes of selected candidate genes per brain region. (D) Most candidate genes of the tectum opticum region had a negative model slope, that is, the more behavioral laterality a fish showed, the relatively higher the gene was expressed in the brain hemisphere facing its prey. (E) In the telencephalon almost all candidate genes had a positive model slope, that is, were relatively down-regulated in the brain side facing the prey. (F) Fewest candidate genes were detected in the hypothalamus, in which slightly more genes had a positive slope than a negative slope.

unexplored so far. Linking asymmetric gene expression in the brain to its functional significance, for example, lateralized behavior, has rarely been investigated and the focus of previous work remains exclusively on humans (Sun et al. 2005; Francks et al. 2007; Johnson et al. 2009; but see Nadler et al. 2006; deCarvalho et al. 2014). To our knowledge, this is the first study to link behavioral laterality to left-right asymmetry in gene expression in different brain regions as well as to neuroanatomical asymmetry, that is, the size differences between the left and right hemispheres.

Our results show that the degree of lateralized behavior in *P. microlepis* reflects the anatomical size differences between

the left and right hemispheric regions in the brain, particularly in the visual center of the brain, the tectum opticum. This paired brain region, which belongs to the mesencephalon, is central in processing visual information and signaling stimuli to other brain regions and motor-neurons (Hubel and Wiesel 1962), involving prey-catching behavior ("orientation toward prey behavior") as documented in salamanders (Himstedt and Roth 1980) and fishes (Al-Akel et al. 1986; Gahtan et al. 2005). It is important to note that, as was shown for other vertebrates, fish brain hemispheres typically control the opposite sides of the individual's body, that is, the right tectum opticum processes incoming visual information

Table 2

List of the Ten Most Significant Candidate Genes in the Paired Brain Regions Including the Tectum Opticum, Telencephalon, and Hypothalamus Based on the Overall Model Fit

	EnsemblProteinID	gene	p-int.	slope	p-var.	r^2
Tectum opticum	ENSONIP00000008842	sncga	0.7479	−0.5608	0.0038	0.5826
	ENSONIP00000014770	rtn4ip1	0.1936	−1.4369	0.0053	0.5518
	ENSONIP00000015841	uhrf1	0.3760	1.7053	0.0065	0.5324
	ENSONIP00000020876	olfml2bb	0.1367	−1.9244	0.0069	0.5274
	ENSONIP00000001535	zgc: 110158	0.4366	0.8602	0.0069	0.5262
	ENSONIP00000025694	zgc: 64106	0.6089	−1.0583	0.0080	0.5115
	ENSONIP00000021527	rbm38 (1 of many)	0.1017	−0.9051	0.0090	0.5001
	ENSONIP00000008777	KCNG3 (1 of many)	0.1829	−1.5335	0.0110	0.4787
	ENSONIP00000012033	NA	0.1464	−0.9119	0.0117	0.4717
	ENSONIP00000018216	zgc: 66014	0.4934	−0.2808	0.0118	0.4708
Telencephalon	ENSONIP00000000966	olfm1a	0.1101	0.7254	0.0039	0.5406
	ENSONIP00000010660	TOB2	0.2427	−0.8450	0.0047	0.5235
	ENSONIP00000002199	cnih2	0.1336	0.5521	0.0074	0.4806
	ENSONIP00000012755	amph	0.1016	0.4358	0.0075	0.4799
	ENSONIP00000012999	arhgef1a	0.1384	0.3386	0.0082	0.4708
	ENSONIP00000006210	alg9	0.1707	0.6165	0.0083	0.4704
	ENSONIP00000004440	prnpb	0.1233	0.3311	0.0086	0.4668
	ENSONIP00000005851	rab3b	0.2061	0.6205	0.0109	0.4425
	ENSONIP00000019106	si: ch211-107o10.3	0.3119	0.5579	0.0127	0.4263
	ENSONIP00000004627	asap2a	0.1627	−1.0733	0.0141	0.4154
Hypothalamus	ENSONIP00000003978	slc7a6	0.1117	1.3624	0.0061	0.5382
	ENSONIP00000004990	brd1b	0.1224	0.9666	0.0081	0.5102
	ENSONIP00000013224	ift20	0.1964	−0.8573	0.0083	0.5086
	ENSONIP00000022752	ldlrb	0.1469	−1.7009	0.0129	0.4610
	ENSONIP00000009502	eps8	0.1512	1.1046	0.0147	0.4463
	ENSONIP00000020992	pappa2	0.1423	3.3755	0.0153	0.4415
	ENSONIP00000003764	dhodh	0.1981	2.0406	0.0191	0.4161
	ENSONIP00000009138	mroh1	0.1397	0.6944	0.0194	0.4143
	ENSONIP00000011249	PKP4 (1 of many)	0.1348	2.5339	0.0196	0.4128
	ENSONIP00000002773	pigt	0.1201	0.8964	0.0198	0.4118

Note.—The table shows the P values of the linear model intercept, the slope estimate, the P values of the slope, the overall model P values and the model r^2. Estimates for log2-fold-changes as a dependent variable.

from the left eye and the left tectum opticum from the right eye via the chiasmus opticum (fig. 1C and D). In fish with strong behavioral laterality, we showed that the tectum opticum hemisphere that is processing the data from the eye that is facing towards the prey during an attack is significantly larger in volume than its counterpart (fig. 2B). In fish with weak behavioral laterality this effect of neuroanatomical left-right asymmetry was not present. These findings are consistent with the patterns previously found in pigeons that the right eye is dominant over the left and accordingly the left hemisphere of their tectum opticum is increased in size (Güntürkün 1997). Our results thus indicate that although the extent of lateralization in behavior varies among individuals, its occurrence may be determined by differences in symmetry of some neuroanatomical regions. This finding also supports the notion that the neurologically more dominant side of the body may be orchestrated by the relatively larger corresponding brain hemisphere (on the contralateral side).

Genome-wide transcriptional profiles of four brain regions analyzed in this study reveal that different brain regions have distinct transcriptional profiles (fig. 3). These results agree with previous findings of regionally specific gene expression patterns in adult mice brain (Nadler et al. 2006; Lein et al. 2007) and also in adult human brain (Hawrylycz et al. 2012). Notably, the cerebellum appears to be transcriptionally the most divergent from the other three brain parts (separated from the others by PC1) as shown for adult mice brain (Nadler et al. 2006), followed by the tectum opticum (separated from the others by PC2) and finally the telencephalon and the hypothalamus appear to be transcriptionally most similar (separated from each other only by PC3). This order corresponds well to their developmental Bauplan and interrelationships, with the telencephalon (most anterior) and the hypothalamus (second most anterior) both being components of the prosencephalon (forebrain), followed by the tectum opticum in the mesencephalon (midbrain) and the cerebellum, a part of the rhombencephalon (hindbrain; figs. 1 and 5). These results
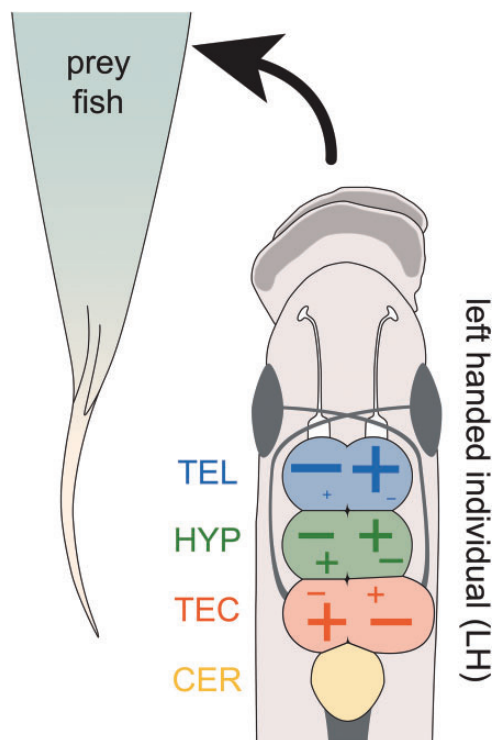
FIG. 5.—Neuroanatomical and transcriptional patterns reflect behavioral laterality in *P. microlepis*. The more lateralized feeding behavior, the larger the hemisphere of the tectum opticum (TEC) that is processing the information of the body side facing the prey fish. Expression differences in the candidate genes between brain hemisphere regions were increased with increasing behavioral laterality. However, the direction of up- (+) or down- (−) regulation in candidate genes showed divergent biases among the brain regions as illustrated. In the telencephalon (TEL), ~85% of candidate genes showed an increase in the hemisphere fold-change with an increasing feeding laterality index (FLI), that is, most genes were relatively up-regulated in the brain hemisphere not facing the prey fish. This trend was less pronounced in the hypothalamus (HYP) where only ~60% of genes were relatively upregulated in the same hemisphere and was completely reversed in the tectum opticum (TEC) where most genes were relatively up-regulated in the hemisphere facing the prey fish.

are consistent with those of previous studies that found that gene expression differences across brain regions recapitulated the developmental processes of the embryonic brain in adult mice based on their cluster analyses (Zapala et al. 2005; Nadler et al. 2006). Likewise, we find a higher number of differentially expressed genes between the cerebellum and any other brain regions, and relatively fewer between the telencephalon and the hypothalamus. The GO enrichment tests on the differentially expressed genes support the transcriptionally distinct position of the cerebellum: comparisons within and between prosencephalic and mesencephalic brain regions resulted in many differentially represented GO categories with each representing only a small fraction of differentially expressed genes (supplementary fig. S3, Supplementary Material online). However, when the other

brain regions are compared with the cerebellum, fewer major "biological process"-GO categories cover most differentially expressed genes, suggesting that the cerebellum is most different from the other brain regions, for example, by substantially differing from each other in genes relating to "integral components of cell membranes", such as ion channel genes.

A central goal of this study was to determine candidate genes that relate to behavioral laterality and we identified genes potentially underpinning behavioral laterality in all three paired brain regions. For the tectum opticum, we found expression differences in 140 genes between the left and right hemispheres that could be related to behavioral lateralization (supplementary table S4, Supplementary Material online). The most significant and promising candidate is synuclein gamma (*sncga*) and is discussed below in more detail. Besides *sncga*, we identified paralogs of other putatively asymmetrically expressed neurotransmitters, such as numerous members of the solute carrier family as well as the glutamate metabotropic receptor family (their functions are summarized in deCarvalho et al. 2014; Karlebach and Francks 2015).

The function of the telencephalon and its role in behaviors in teleost fishes remain largely unstudied. The telencephalon, known as the cognitive center of the vertebrate brain, is thought to be involved in processing all sensory information especially olfactory signals coming from the olfactory bulbs, learning, directing active movements and social-reproductive behaviors (Overmier and Patten 1982; Butler and Hodos 2005). Lesion studies have suggested that the telencephalon of the teleost fish plays a role in spatial learning and memory (Kaplan and Aronson 1967; reviewed in Salas et al. 2006). We identified a pronounced bias in the slope of candidate genes relating to lateralized feeding behavior in *P. microlepis*, indicating that ~85% of these genes are up-regulated in the telencephalon hemisphere away from prey fish and thus putatively controlling the body side of the scale-eater facing the prey fish. Among these telencephalic candidate genes, interestingly, we found *mgrn1b* that has been shown to crucially affect left-right patterning in mice and that is important for proper neuronal development (Cota et al. 2006). *Lrrtm1*, another candidate gene, has been suggested to be related to handedness and schizophrenia in humans (Francks et al. 2007). Changes in the expression of the telencephalic candidate *kcng3*, a gene coding for a potassium channel subunit, might be related to handedness, as changes in the closely related *kcns3* have been suggested to be related to schizophrenia in humans (Georgiev et al. 2014). Schizophrenia is, interestingly, a condition that is associated with reduced brain lateralization and handedness (Sommer et al. 2001). Expression of two protocadherins (*pcdh1b* and *pcdh17*) was found to be significantly associated with lateralized behavior, a gene family that has been suggested to be involved with the morphological asymmetry in *P. microlepis* from a population genomics study (Raffini et al. 2016). Other candidates include
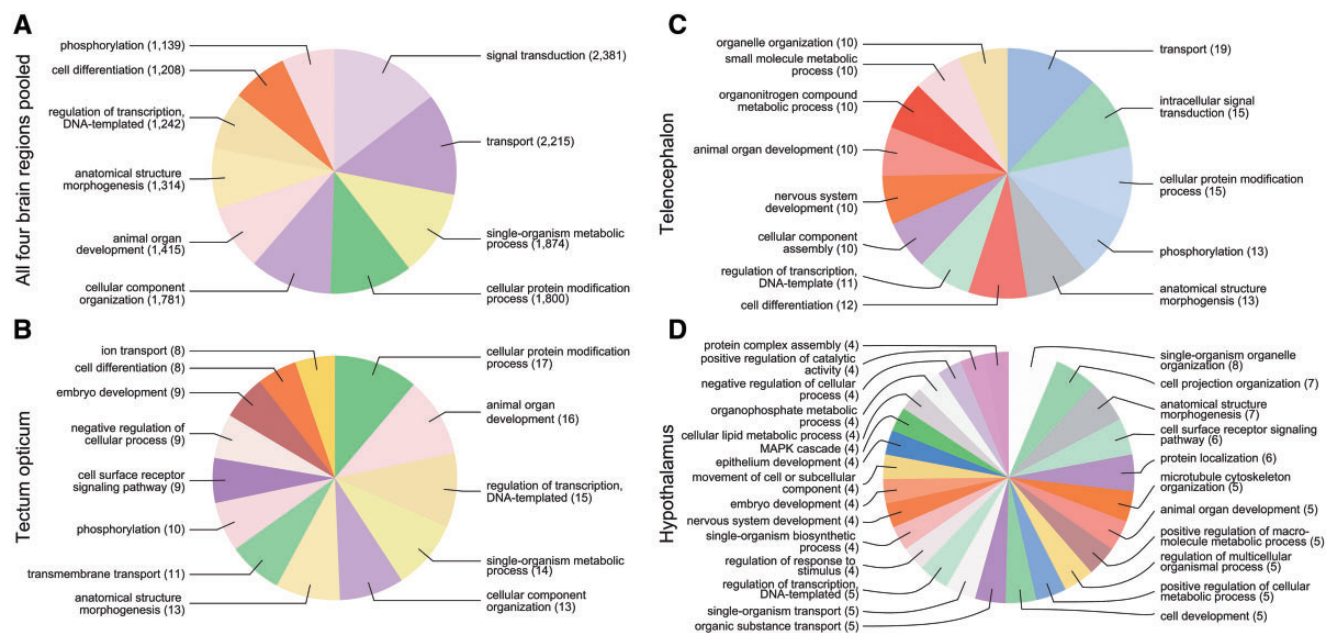
Fig. 6.—Gene ontology (GO) term analysis on biological processes of all genes (A) pooled across all four brain regions, and on genes with a linear relationship to behavioral laterality in the paired (B) tectum opticum, (C) telencephalon, and (D) hypothalamus. GO term composition suggests that laterality candidate genes belong to different biological processes and their composition is brain region specific.

cadm1b, cadpsb, grm2, and kctd2, which themselves or their paralogs have been reported to be expressed asymmetrically in the brain of zebrafish (deCarvalho et al. 2014). Interestingly, grm2, that had a significant negative slope in the tectum opticum, had a positive slope in the telencephalon. It thus exemplifies the overall inversion of slope biases that we observed between the two brain regions (figs. 4 and 5) that may be due to the circumstance that the tectum opticum is processing visual signals from the eye of the opposite body side while the telencephalon processes olfactory signals from the same body side.

The smallest number of candidate genes with the most subtle slope bias were detected in the hypothalamus, the brain region that lies ventrally, and developmentally between the tectum opticum and the telencephalon. Identified candidate genes include smad2, which is crucial in early embryonic development of asymmetric structures (Tremblay et al. 2000) and members of the solute carrier family (slc7a6, slc23a1, and slc33a1). In general, the hypothalamus shows the least asymmetry in gene expression in the paired brain regions that we analyzed.

Highlighting our overall evidence, we found strong support for behavioral laterality represented in the tectum opticum by its neuroanatomical and transcriptional asymmetries, as our results show a left-right size difference and also a pronounced bias in the linear model slopes in this particular brain region. The tectum opticum-specific candidate genes appear to be downregulated in the brain hemisphere that controls the body side facing the prey fish during an attack—the opposite

trend we find in the telencephalon (fig. 5). Although the tectum opticum is processing signals from the eye on the opposite side of the body, the telencephalon processes information from the olfactory sensors on the same body side. These two findings combined may explain the inverted biases of gene expression patterns between these two brain regions (figs. 4 and 5). The hypothalamus is a part of the diencephalon, which is thought to be the most complex region of the teleost brain (Cerdá-Reverter et al. 2001). Among other functions, it processes information of gustatory information, vision, olfaction and reproduction (Braford and Northcutt 1983; Northcutt and Wullimann 1988; Wullimann 1997). The neuroanatomical and functional diversities of the hypothalamus is probably also reflected in the most diverse set of GO terms that deviated from the reference set (fig. 6).

One of the exciting findings in our study is the highly significant correlation between the degree of asymmetrical expression in sncga and that of behavioral lateralization. sncga belongs to a group of soluble proteins (synucleins) that act as neurotransmitters and are commonly expressed in neural tissues; however, sncga is typically expressed in peripheral neurons and the retina (George 2002). In zebrafish, sncga was found to be asymmetrically expressed in the habenular nuclei—a diencephalic complex that has been shown to be involved in motor behaviors and the motivation to exhibit them, and that is thought to be a hub for asymmetry in the vertebrate brain (Bianco and Wilson 2009; Chen et al. 2009; Ichijo et al. 2017). Specifically, habenular nuclei are shown to signal motor neurons and interact with the dopaminergic

system, potentially leading to behavioral side preferences (reviewed in Bianco and Wilson 2009; Gutiérrez-Ibáñez et al. 2011; as suggested for *P. microlepis* by Ichijo et al. 2017). This is an exciting hypothesis that deserves further studies.

The causal relationships leading to the lateralized behavioral phenotypes in *P. microlepis* may be critically influenced by the brain anatomical and transcriptional asymmetries that we identified. Fish with structural and functional asymmetries likely controlled by a genetic component (Ichijo et al. 2017) may be stimulated to exhibit lateralized behaviors, such as feeding. Lateralized foraging behavior finally may lead to plastic changes in the oral jaw phenotype in *P. microlepis* (Van Dooren et al. 2010; Palmer 2012; Lee et al. 2012). Phenotypic plasticity has been shown to be a prominent feature of many cichlid fishes (e.g., Meyer 1987; Klingenberg et al. 2003; Muschick et al. 2011; Schneider and Meyer 2017) possibly as a developmental basis for both the diversity of jaw shapes and the lateralized jaws for which this species is renowned. However, previous studies seemed to show that the jaw laterality is already present in *P. microlepis* fry before they start actively to feed, arguing against plasticity (Hori 1993; Stewart and Albertson 2010). Our more recent studies reconciled the disputed mechanisms for morphological asymmetry where not only genes, but also environmental effects appear to be responsible for the observed variation in this trait (Lee et al. 2015; Raffini et al. 2016). To more fully resolve this issue, time-course studies investigating both morphological asymmetries in the jaw and the brain as well as differential gene expression in the brain starting at the earliest possible time-point and covering the time until clear feeding preferences (e.g., at an age of 2-months; Lee et al. 2012) would be necessary. Such studies would allow to determine the developmental sequence of asymmetry in the jaw and brain, and also behavior (Ichijo et al. 2017).

Some of our earlier work (Lee et al. 2012) and results from the current analysis suggest that behavioral laterality may occur earlier than morphological laterality in the jaw or that both may emerge independently, given only a weak correlation between mouth asymmetry and behavioral laterality in "laboratory-reared" scale-eating cichlids. We also found that in laboratory-reared juvenile fish (e.g., at an age of 2-months) that had been raised on *Artemia* nauplii and flake food, the degree of mouth asymmetry was relatively small based on our quantitative measurements, although we could document an early strong laterality in behavioral preference when feeding on scales (Lee et al. 2012). These findings suggest that lateralized foraging behavior might precede and "guide" morphological asymmetry via plasticity. A central question that follow-up studies should thus ask is how strongly behavioral laterality is genetically determined (and heritable). Whether the asymmetry of brain regions, particularly tectum opticum (as shown in this study) is a direct consequence of genes that cause lateralized behaviors or whether these brain regions respond to lateralized behaviors secondarily remains unclear. However, the former scenario is more plausible, given that the test fish used for brain anatomical and transcriptional asymmetries analyzed here had not had an opportunity to feed on scales from prey fish before.

Perissodus microlepis is an evolutionary text-book example of negative frequency-dependent selection due to its iconic mouth asymmetry and feeding laterality. Despite prior efforts to elucidate the genetic or environmental bases of morphological mouth asymmetry (e.g., Lee et al. 2015; Raffini et al. 2016), relatively little attention had been paid to the molecular basis of behavioral laterality so far. In this study we show that feeding laterality in *P. microlepis* is reflected in its neuroanatomy, particularly in the tectum opticum. Transcriptional profiles further suggest that in both the tectum opticum and telencephalon, gene expressions are also lateralized, and that these gene expression lateralities are likely to be causally linked to behavioral laterality. Given the observed neuromolecular basis of behavioral laterality, the next work needs to focus on functional tests for the proposed causal relationships and on the heritability of the neuroanatomical and transcriptional asymmetries.

Authors' Contributions

H.J.L. and A.M. designed the study, H.J.L. carried out fish breeding, H.J.L. and E.L. conducted foraging preference experiments and brain volume measurements. J.H.K. and P.F. performed RNA experiments, R.F.S., T.M., and P.F. analyzed and interpreted the transcriptomic data. H.J.L., R.F.S., T.M., J.H.K., P.F., and A.M. drafted the manuscript. All authors edited and agreed to a final version of the manuscript.

Supplementary Material

Supplementary data are available at *Genome Biology and Evolution* online.

Acknowledgments

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