

Reporting Summary

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Statistical parameters

When statistical analyses are reported, confirm that the following items are present in the relevant location (e.g. figure legend, table legend, main text, or Methods section).

n/a | Confirmed

- ☐ ☒ The exact sample size (*n*) for each experimental group/condition, given as a discrete number and unit of measurement
- ☐ ☒ An indication of whether measurements were taken from distinct samples or whether the same sample was measured repeatedly
- ☐ ☒ The statistical test(s) used AND whether they are one- or two-sided
Only common tests should be described solely by name; describe more complex techniques in the Methods section.
- ☐ ☒ A description of all covariates tested
- ☐ ☒ A description of any assumptions or corrections, such as tests of normality and adjustment for multiple comparisons
- ☐ ☒ A full description of the statistics including central tendency (e.g. means) or other basic estimates (e.g. regression coefficient) AND variation (e.g. standard deviation) or associated estimates of uncertainty (e.g. confidence intervals)
- ☐ ☒ For null hypothesis testing, the test statistic (e.g. *F*, *t*, *r*) with confidence intervals, effect sizes, degrees of freedom and *P* value noted
Give P values as exact values whenever suitable.
- ☒ ☐ For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings
- ☒ ☐ For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes
- ☒ ☐ Estimates of effect sizes (e.g. Cohen's *d*, Pearson's *r*), indicating how they were calculated
- ☐ ☒ Clearly defined error bars
State explicitly what error bars represent (e.g. SD, SE, CI)

Our web collection on [statistics for biologists](#) may be useful.

Software and code

Policy information about [availability of computer code](#)

Data collection

NA

Data analysis

Quality control: FastQC. v.0.11.4. 553 (www.bioinformatics.babraham.ac.uk/projects/fastqc)
de novo assembly: Trinity v2.2, RSEM v1.2.20, Transdecoder (Transdecoder v3.0.1, <http://transdecoder.github.io>).
Genome-guided assembly: HiSat 2.0.5 - Stringtie v1.3.2 suite
Reference transcriptome construction: CD-Hit-Est
Differential expression: RSEM v1.2.20, DESeq2

For manuscripts utilizing custom algorithms or software that are central to the research but not yet described in published literature, software must be made available to editors/reviewers upon request. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Research [guidelines for submitting code & software](#) for further information.

Data

Policy information about [availability of data](#)

All manuscripts must include a [data availability statement](#). This statement should provide the following information, where applicable:

- Accession codes, unique identifiers, or web links for publicly available datasets
- A list of figures that have associated raw data
- A description of any restrictions on data availability

Normalized counts for all groups of differentially expressed genes as well as all expressed genes are available as Supplementary Datasets. RNA reads have been deposited at the NCBI Sequencing Read Archive, BioProject ID PRJNA413692. Additional data may be requested from the authors.

Field-specific reporting

Please select the best fit for your research. If you are not sure, read the appropriate sections before making your selection.

☒ Life sciences ☐ Behavioural & social sciences ☐ Ecological, evolutionary & environmental sciences

For a reference copy of the document with all sections, see [nature.com/authors/policies/ReportingSummary-flat.pdf](https://www.nature.com/authors/policies/ReportingSummary-flat.pdf)

Life sciences study design

All studies must disclose on these points even when the disclosure is negative.

Sample size	270 samples total, divided into 54 pools (27 pools for each the optic tectum and the telencephalon). We performed our experiment in 3 lines (WT, LB and SB), conducting 3 treatments for each line. For each line/treatment combination in our experiment, we had 15 replicate females, divided into 3 pools. This is equivalent to 9 pools for each line and 9x3=27 pools, with 5 female brains per pool, 135 females. For each female we dissected the optic tectum and telencephalon separately for sequencing.
Data exclusions	Some samples were clear outliers when considering overall expression and were thus excluded from the analysis. These samples included 2 optic tectum samples and 1 telencephalon sample as detailed in the supplementary materials.
Replication	For each treatment within each line, we have 3 pools composed of 5 individual females each (135 females total). Moreover we have used two separate lines with the same preference phenotype corresponding to 2 independent biological replicates for the preference phenotype.
Randomization	Within each line, focal females were chosen at random and randomly matched with the different presentation males. Focal females belonging to same replicate selection line and the same treatment were presented with different males to avoid uncontrolled male-driven changes in expression. For this, we balanced the number of large-brained, small-brained and wild-type females presented to colorful males, dull males and females respectively per day
Blinding	Investigators were blind to group allocation and data collection. Moreover, trials were videotaped so researchers were not in the room while behavioral trials were conducted to avoid interference.

Reporting for specific materials, systems and methods

Materials & experimental systems

n/a	Involved in the study
☒	☐ Unique biological materials
☒	☐ Antibodies
☒	☐ Eukaryotic cell lines
☒	☐ Palaeontology
☐	☒ Animals and other organisms
☒	☐ Human research participants

Methods

n/a	Involved in the study
☒	☐ ChIP-seq
☒	☐ Flow cytometry
☒	☐ MRI-based neuroimaging

Animals and other organisms

Policy information about [studies involving animals](#); ARRIVE guidelines recommended for reporting animal research

Laboratory animals

Guppies used in our experiment are laboratory-raised descendants of Trinidad guppies sampled from the high predation populations of the Quare River (Trinidad). We based our study on guppies from this wild-type population and two different selection lines, derived from the wild-type fish, which have been selected on relative brain size. In summary fish were indirectly

selected based on parental brain size achieving a difference of up to 13.6% in relative brain size between lines selected to have small brains, here denoted small brain lines (SB lines), and lines selected to have larger brain (LB lines). Three replicate lines were generated for each selection regime.

FOCAL FEMALES: For this study, we used virgin females from the fifth generation of selection, all aged approximately 6 months. We used a total of 135 females, 45 from each line.

At the end of each trial females were euthanized by transfer to ice water. After 45 seconds, and with aid of a Leica S4E microscope, we removed the top of the skull to expose the brain.

PRESENTATION MALES and FEMALES: For our study we divided females among 3 treatments: two treatments represented a male evaluation context, in which females were presented either an attractive male (attractive treatment) or an unattractive male (dull treatment), and a third treatment in which females were exposed to another female representing a general social interaction treatment. We used 30 presentation males selected for their colorful or dull patterns. Presentation females were chosen at random from the wild-type population. Presentation animals were reintroduced into their original laboratory populations after the experiments.

The experiment was performed in accordance with ethical applications approved by the Stockholm Ethical Board (Reference number: N173/13, 223/15 and N8/17). These applications are consistent with the Institutional Animal Care and Use Committee guidelines

Wild animals

The study did not involve wild animals

Field-collected samples

The study did not involve field-collected samples