Supplementary Information

Early neurogenomic response associated with variation in guppy female mate preferences

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This PDF file includes:

- Materials and Methods
- Figs. S1 to S8
- Tables S1 to S11
- Captions for databases S1 to S2
- Supplementary references

Other Supplementary Materials for this manuscript includes the following:

Databases S1 to S2 as zipped archives:

1. Databases S1: Optic tectum normalized count data for differentially expressed genes
2. Databases S2: Telencephalon normalized count data for differentially expressed genes

Figure S1: Methodological overview of differentially expressed gene identification. The same procedure was carried out in Preference lines and Non-preference lines in parallel. Of all significant DE genes for each pairwise comparison, we retained only those with concordant changes in expression in all replicate samples (i.e. either increase in expression between treatment 1 and treatment 2 in ALL samples as illustrated in the graph inset, or decrease in expression between treatments in ALL samples). Once we determined the final set of DE genes for each pairwise treatment comparison, we defined Preference DE genes (or Non-preference DE genes in Non-preference lines), and Social DE genes as described in the lower Venn diagrams.

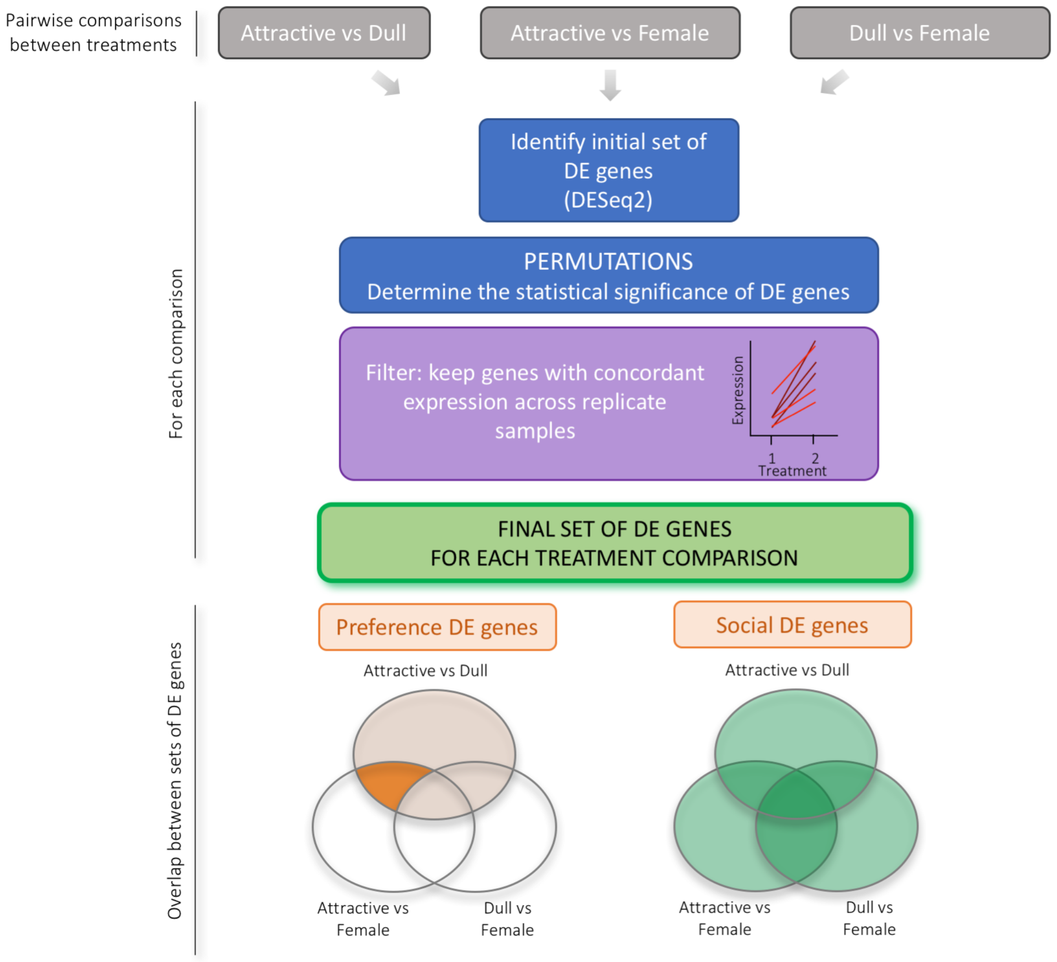


Figure S2: Heatmap of normalized expression correlations between samples. Upper half of heatmap corresponds to optic tectum (n= 20396 expressed transcripts) and lower half to telencephalon (n=19571 expressed transcripts). Dendrograms illustrate sample hierarchical clustering based on sample expression distance. Outliers removed from analyses are indicated with (*).

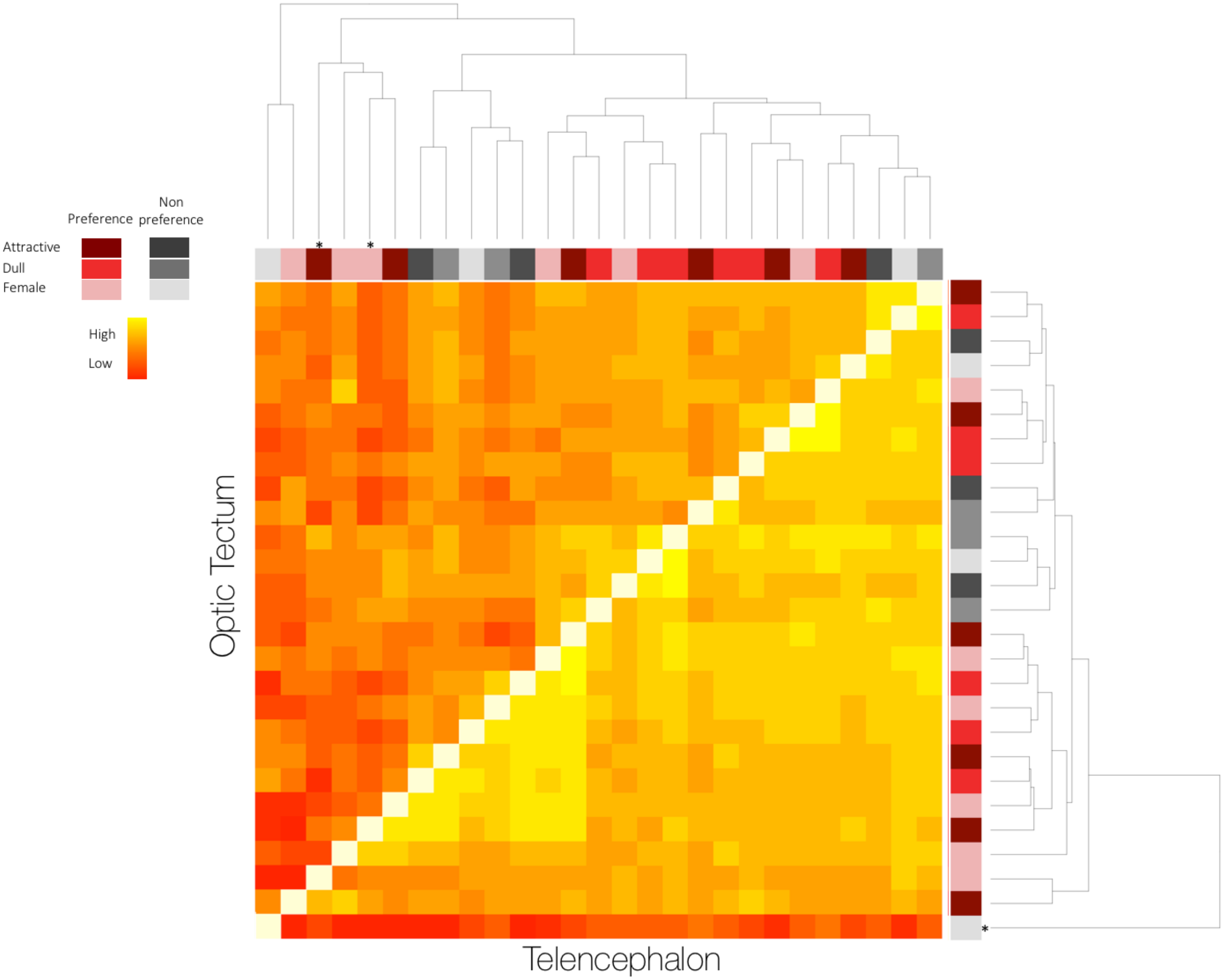
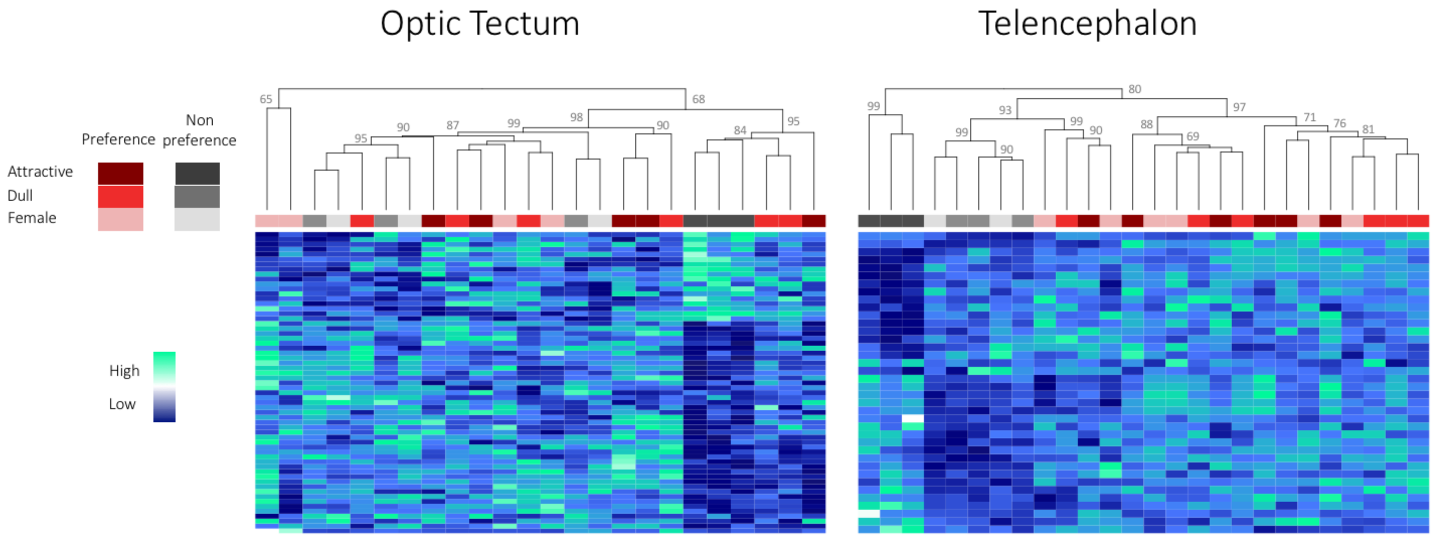


Figure S3: Hierarchical gene-expression clustering of Non-preference DE genes.
Clustering of samples for Non-preference DE genes, differentially expressed between attractive and dull male treatments in Non-preference females. We found 61 Non-preference DE genes in the optic tectum and 38 Non-preference DE genes in the telencephalon. Colors below dendrogram correspond to sample treatment and line as indicated in the legend. Values on top of nodes correspond to bootstrap Approximately Unbiased p-values[1], computed by multiscale bootstrap resampling (all bootstrap values >68%, some not shown for clarity).


Optic Tectum

Telencephalon

Figure S4: Co-expression networks. Overview of optic tectum (A) and telencephalon (B) co-expression networks. Genes highlighted in networks correspond to Preference DE genes in red, Non-preference DE genes in grey, known preference/social behavior genes in green, synaptic plasticity genes & immediate early genes in black/black edge.

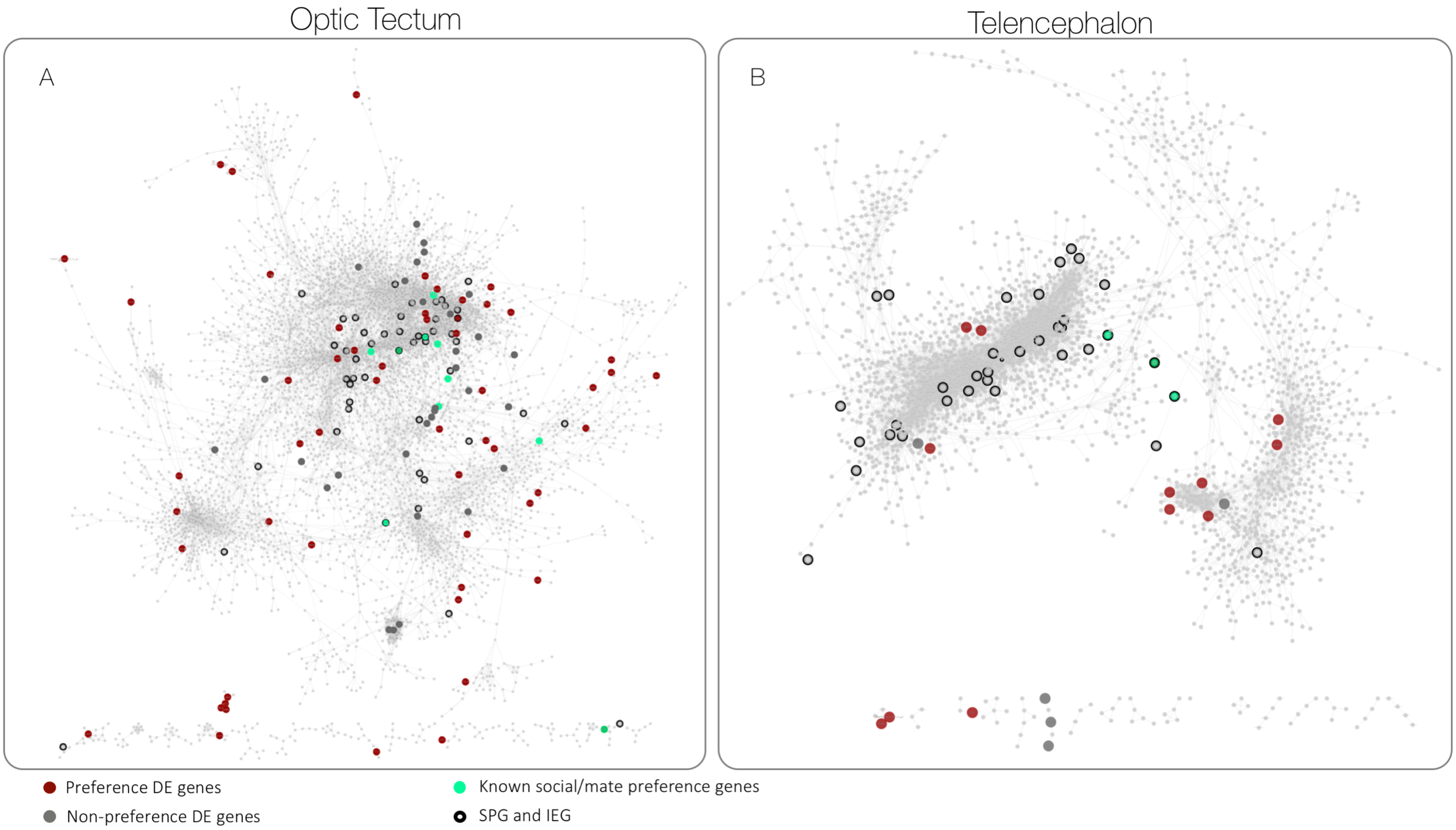
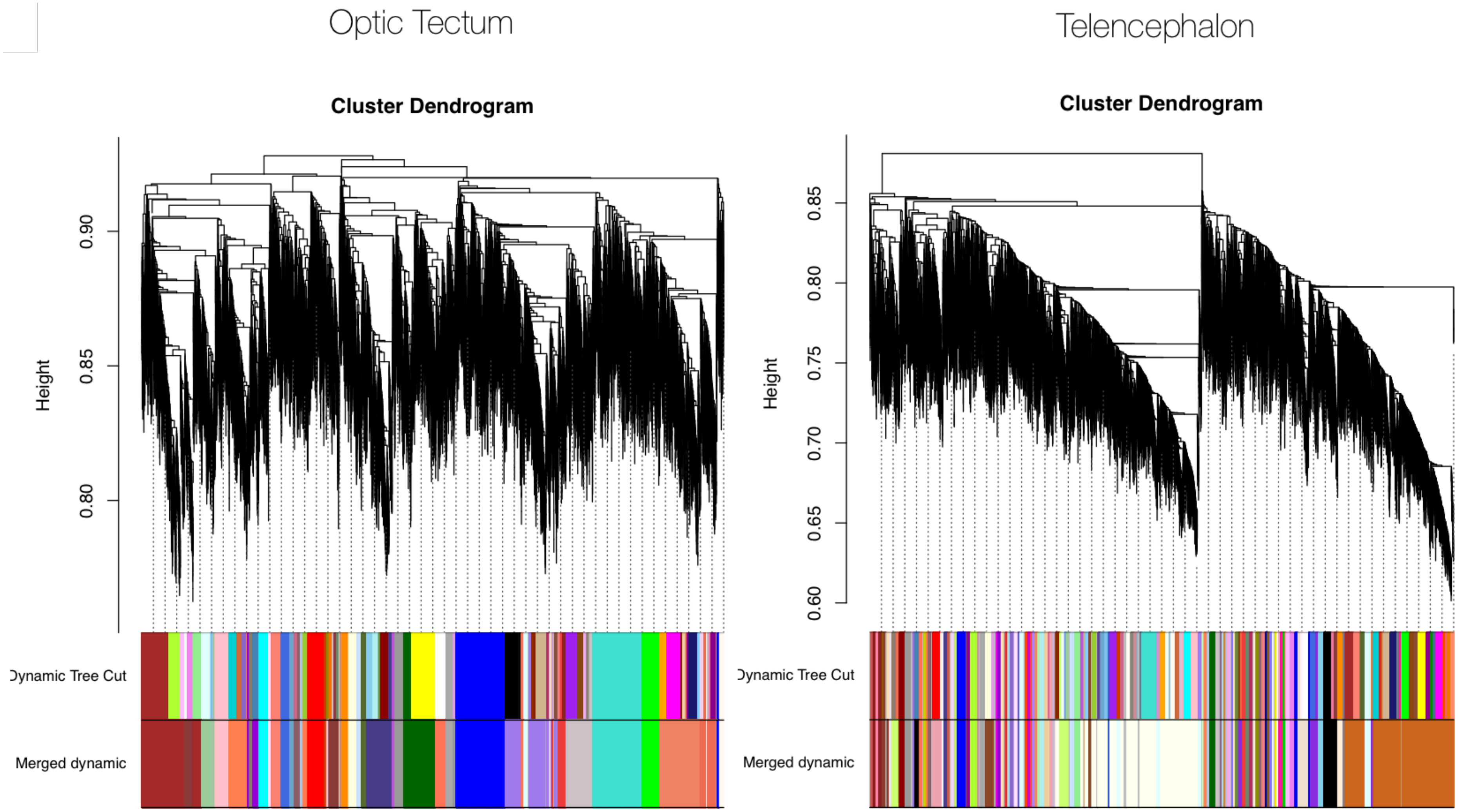


Figure S5: Co-expression gene module identification dendrograms. Gene dendrograms showing the co-expression modules identified by the WGCNA dynamic tree cut function. Top colors correspond to module color labels before merging and bottom colors after merging modules whose expression profiles are very similar. Co-expression similarity between modules was estimated by calculating module eigengene correlations.



Optic Tectum

Telencephalon

Figure S6: Social DE genes expression change between attractive and dull treatments in Preference lines relative to Non-preference lines. Scatterplots showing two different expression patterns of Social DE genes: Scatterplots of attractive:dull $\log_2$ fold-change in Preference lines (x-axis) against Non-preference lines (y-axis) for Social DE genes in the optic tectum (left) and telencephalon (right). Darker blue points correspond to higher $\log_2$ fold-change values. In the optic tectum, the outlier gene GOPC was omitted from the graph to better visualize differences across genes. For this gene, $\log_2$ fold-change in Preference lines is -4.61 and $\log_2$ fold-change in Non-preference lines is 3.45. Social DE genes could either play similar functions modulating social interactions in both Preference and Non-Preference lines, in which case we would expect them to change in the same direction between the attractive and dull male treatments in both line types. We found 174 optic tectum and 73 telencephalon genes fit this pattern (Fig. S6, quadrants I and III). In contrast, we would expect Social DE genes associated with the loss of female preference phenotype in Non-preference lines would show the opposite pattern. Consistent with this expectation, we identified 213 optic tectum and 88 telencephalon genes that changed in opposite directions between the attractive and dull male treatments in both Preference and Non-Preference lines (Fig. S6, quadrants II and IV).

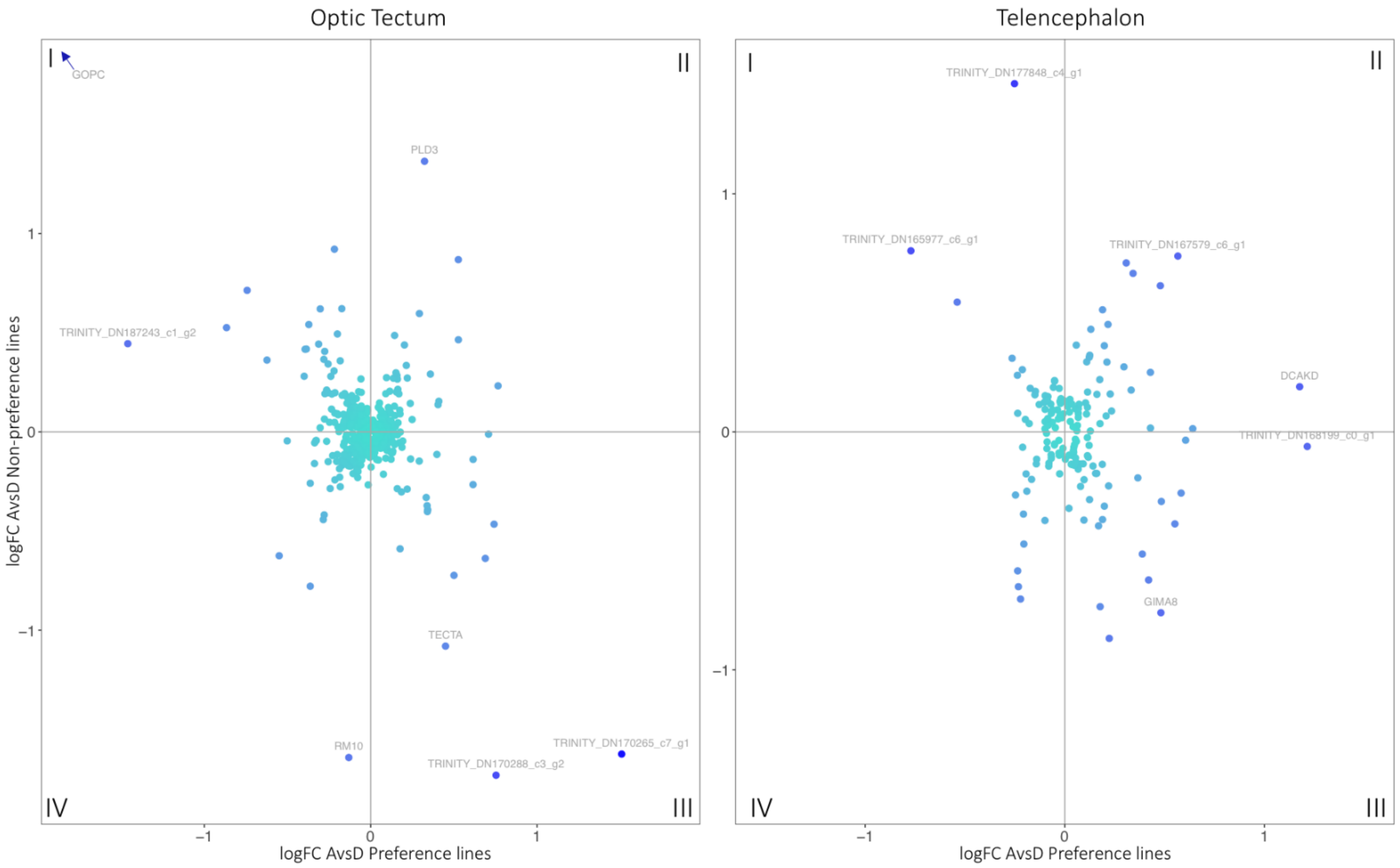


Figure S7: Gene expression for Transcription factors among Social DE genes known to have TF motifs among Preference DE genes. Plots showing expression differences by treatment and line for transcription factors in Social DE genes found to have regulatory motifs among Preference DE genes for optic tectum (A) and telencephalon (B). Points indicate mean for treatment group and whiskers extend to minimum and maximum values. Significant pairwise treatment comparisons (based on GLM permutations within Preference lines and Non-preference lines) denoted with (*).

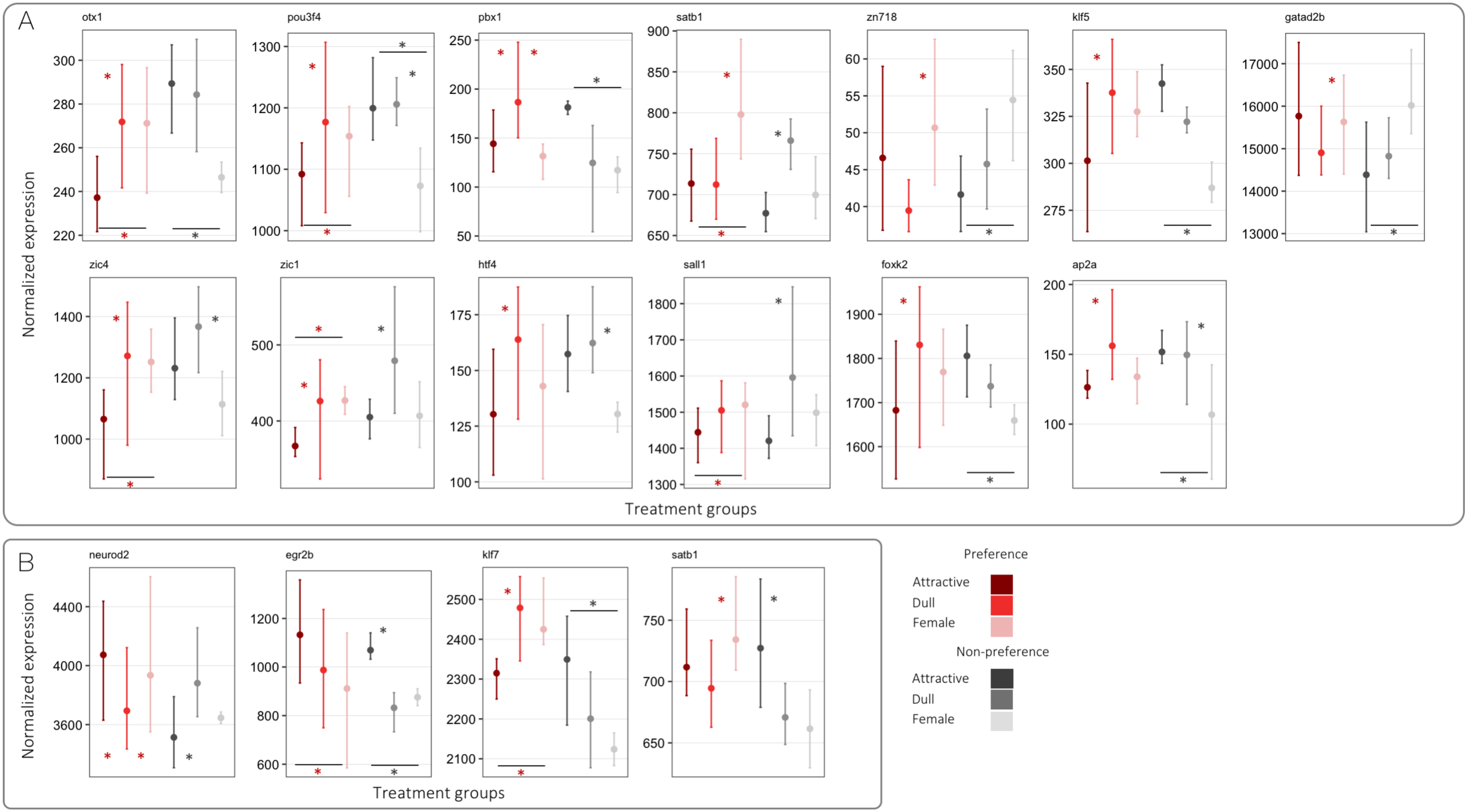


Figure S8: Transcription factors motifs enriched among Preference DE genes. Transcription factor motifs associated with Preference DE genes in the optic tectum (A) and the telencephalon (B). TRANSFAC transcription factor binding sites collection as implemented in g:Profiler was used to determine TF motifs associated with each gene (FDR multiple testing correction and a p-value<0.05). Only transcription factors relevant to social behavior, synaptic plasticity or belonging to the same protein families as genes identified in social behavior studies were included. Color of grid is proportional to the number of genes within each module with the corresponding transcription factor motif.

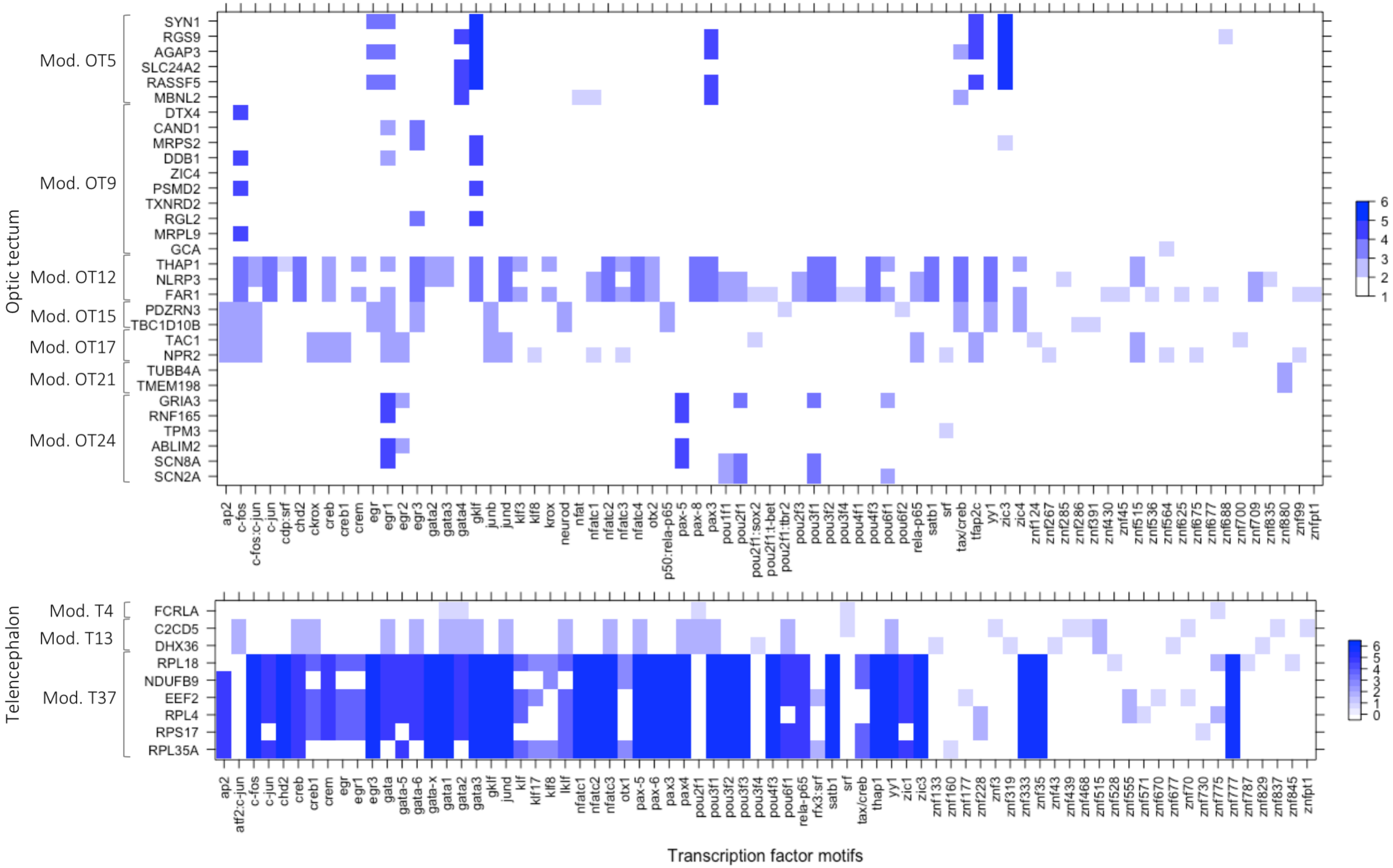


Table S1: Identity of in-network Preference DE genes.

OPTIC TECTUM			
Gene name	Module	Gene description	Relevant pathways - functions - phenotypes$^{\delta}$
Preference DE genes			
trinity_dn187470_c3_g1	OT3	N/A	N/A
agap3	OT5	ArfGAP with GTPase domain, ankyrin repeat and PH domain 3 [Source:ZFIN;Acc:ZDB-GENE-110927-1]	response to stimulus - signal transduction
mbnl2	OT5	Muscleblind-like protein 2	regulation of gene expression
rassf5	OT5	Ras association domain family member 5 [Source:HGNC Symbol;Acc:HGNC:17609]	response to stimulus- signaling - signal transduction ras signaling pathway
rgs9	OT5	regulator of G protein signaling 9 [Source:HGNC Symbol;Acc:HGNC:10004]	response to stimulus - response to estrogen- visual perception- regulation of response to stimulus- G-protein coupled receptor signaling pathway- phototransduction pathway
slc24a2	OT5	solute carrier family 24 (sodium/potassium/calcium exchanger), member 2 [Source:ZFIN;Acc:ZDB-GENE-060825-277]	synaptic signaling - response to stimulus - behavior - cognition - learning and memory - visual perception - calcium ion transport- regulation of synaptic plasticity- long-term synaptic potentiation - ion channel activity
syn1	OT5	synapsin I [Source:ZFIN;Acc:ZDB-GENE-081105-93]	synaptic signaling - neurotransmitter release - vesicle-mediated transport in synapse- Specific learning disability - aggressive behavior - autistic behavior- serotonin and dopamine neurotransmitter release cycle
ctc1	OT6	CTS telomere maintenance complex component 1 [Source:ZFIN;Acc:ZDB-GENE-061103-271]	response to stimulus - abnormality of vision
zacrp4	OT9	C1q and TNF related 4	N/A
cand1	OT9	cullin-associated and neddylation-dissociated 1 [Source:ZFIN;Acc:ZDB-GENE-040426-2872]	response to stimulus
ddb1	OT9	damage specific DNA binding protein 1 [Source:HGNC Symbol;Acc:HGNC:2717]	response to stimulus - detection of stimulus - signal transduction
dtx4	OT9	deltex 4, E3 ubiquitin ligase [Source:ZFIN;Acc:ZDB-GENE-070410-129]	signal transduction - Notch signaling pathway
gca	OT9	Grancalcin	response to stimulus
mrpl9	OT9	mitochondrial ribosomal protein L9 [Source:ZFIN;Acc:ZDB-GENE-070717-4]	gene expression - translation
mrps2	OT9	mitochondrial ribosomal protein S2 [Source:ZFIN;Acc:ZDB-GENE-070112-992]	gene expression - translation
psmd2	OT9	proteasome 26S subunit, non-ATPase 2 [Source:HGNC Symbol;Acc:HGNC:9559]	signal transduction - wnt signaling pathway- MAPK cascade - TCF dependent signaling in response to Wnt - Signaling to RAS - Gastrin-CREB signaling pathway via PKC and MAPK
rgl2	OT9	ral guanine nucleotide dissociation stimulator-like 2 [Source:ZFIN;Acc:ZDB-GENE-010131-1]	signal transduction - response to stimulus - Ras protein signal transduction - Rho GTPase binding - Ras signaling pathway
txnrd2	OT9	Thioredoxin Reductase 2	response to stimulus
zic4	OT9	zic family member 4 [Source:ZFIN;Acc:ZDB-GENE-040622-4]	cation binding - brain development
far1	OT12	Fatty acyl-CoA reductase 1	primary metabolic process- abnormality of the nervous system
nlrp3	OT12	NLR Family Pyrin Domain Containing 3	detection of biotic stimulus - signal transduction - Behavioral abnormality
thap1	OT12	THAP domain containing, apoptosis associated protein 1 [Source:ZFIN;Acc:ZDB-GENE-060519-9]	abnormality of the nervous system
hk1	OT14	Hexokinase 1	NADH metabolic process - abnormality of vision
pdzrn3	OT15	(PDZ Domain Containing Ring Finger 3	synapse organization
tbc1d10b	OT15	TBC1 Domain Family Member 10B	Ras GTPase binding - localization
npr2	OT17	Natriuretic Peptide Receptor 2	sexual reproduction - signaling - signal transduction - female gamete generation - regulation of oogenesis - signal transducer activity - oxytocin signaling pathway
tac1	OT17	tachykinin 1 [Source:ZFIN;Acc:ZDB-GENE-060503-904]	Reproduction - cognition - sensory perception - synaptic transmission, GABAergic- behavior - learning and memory - associative learning - long-term memory - detection of stimulus - glucocorticoid and corticosterone secretion - neuropeptide signaling pathway - sexual

			reproduction - MATING - copulation - Gastrin-CREB signaling pathway via PKC and MAPK - Signaling by GPCR
trinity_dn186071_c6_g2	OT17	N/A	N/A
otx1b	OT18	orthodenticle homeobox 1b [Source:ZFIN;Acc:ZDB-GENE-980526-400]	gene expression
tubb4a	OT21	Tubulin beta chain	cellular localization - GnRHR signaling
tmem198	OT21	Transmembrane Protein 198	positive regulation of Wnt signaling pathway - regulation of signal transduction - regulation of response to stimulus - GnRHR signaling
baz2a	OT22	bromodomain adjacent to zinc finger domain, 2A [Source:ZFIN;Acc:ZDB-GENE-041010-202]	regulation of gene expression - gene silencing -
loc103458058	OT22	uncharacterized LOC103458058 [Poecilia reticulata (guppy)]	N/A
ablim2	OT24	Actin Binding LIM Protein Family Member 2	gene expression
gria3	OT24	glutamate receptor, ionotropic, AMPA3 (alpha 3) [Source:MGI Symbol;Acc:MGI:95810]	ion transmembrane transport - synaptic signaling- signal transduction - glutamate receptor signaling/ AMPA glutamate receptor activity - pathway - gated channel activity - neurotransmitter receptor activity - Intellectual disability, severe - behavioral abnormalities - Abnormal emotion/affect behavior - autistic behavior - Long-term depression- Glutamatergic synapse- cAMP signaling pathway - Activation of NMDA receptor upon glutamate binding and postsynaptic events
loc108166223	OT24	uncharacterized LOC108166223 [Poecilia reticulata (guppy)]	N/A
rnf165	OT24	Ring Finger Protein 165	response to external stimulus - signal transduction - transmembrane receptor protein serine/threonine kinase signaling pathway - regulation of gene expression - regulation of BMP signaling pathway
rtbs	OT24	Putative RNA-directed DNA polymerase from transposon BS	N/A
scn2a	OT24	sodium channel, voltage-gated, type II, alpha [Source:MGI Symbol;Acc:MGI:98248]	ion transmembrane transport - response to stimulus - signal transduction - voltage-gated channel activity - abnormality of vision - abnormality of higher mental function - Cognitive impairment - Memory impairment - Behavioral abnormality
scn8a	OT24	sodium voltage-gated channel alpha subunit 8 [Source:HGNC Symbol;Acc:HGNC:10596]	regulation of transmembrane transport - voltage-gated channel activity - Behavioral abnormality -short attention span - autistic behavior - Cognitive impairment - Memory impairment - Intellectual disability - abnormality of vision
tpm3	OT24	tropomyosin 3 [Source:ZFIN;Acc:ZDB-GENE-030826-16]	abnormality of the eye - Pathways in cancer
col9a3	OT26	Collagen Type IX Alpha 3 Chain	reproduction and reproductive process - abnormality of vision and the eye - ECM-receptor interaction
dhrs11	OT34	dehydrogenase/reductase (SDR family) member 11a [Source:ZFIN;Acc:ZDB-GENE-060929-324]	oxidoreductase activity
ager	OT34	Advanced glycosylation end product-specific receptor	gene expression - response to stimulus - signal transduction - MAPK cascade - JNK cascade - p38MAPK cascade - long term synaptic depression - regulation of long term synaptic depression - positive regulation of JUN kinase activity
ubtf	OT34	Upstream Binding Transcription Factor, RNA Polymerase I	gene expression
alpl	OT36	alkaline phosphatase, liver/bone/kidney [Source:ZFIN;Acc:ZDB-GENE-040420-1]	reproduction and reproductive process - response to stimulus - response to steroid hormone and corticosteroid (glucocorticoid) - hydrolase activity - abnormality of the eye
gpatch8	OT36	G patch domain containing 8 [Source:ZFIN;Acc:ZDB-GENE-030131-6966]	RNA binding - protein binding- ion binding
axin2	OT40	axin 2 [Source:HGNC Symbol;Acc:HGNC:904]	regulation of hydrolase activity - signaling - localization - response to stimulus - signal transduction - Wnt signaling pathway - regulation of gene expression - positive regulation of signal transduction - Pathways in cancer
cask	OT40	Calcium/Calmodulin Dependent Serine Protein Kinase	gene expression - response to external stimulus - localization - ion transport - purine nucleoside metabolic process- synaptic signaling - regulation of neurotransmitter levels - presynaptic process involved in chemical synaptic transmission - neurexin family protein binding - abnormality of the eye and optic nerve - abnormality of vision
dnaja3	OT40	DnaJ Heat Shock Protein Family (Hsp40) Member A3)	immune system process - gene expression - synapse organization - response to stimulus - signal transduction - ion binding - purine nucleotide binding

kirrel2	OT40	Kin Of IRRE Like	primary metabolic process
mrpl38	OT40	mitochondrial ribosomal protein L38 [Source:ZFIN;Acc:ZDB-GENE-040426-2373]	primary metabolic process - gene expression - translation
otx1	OT40	orthodenticle homeobox 1 [Source:MGI Symbol;Acc:MGI:97450]	gene expression - sensory organ development - sensory organ morphogenesis - nucleic acid binding transcription factor activity
pbx1	OT40	pre-B-cell leukemia homeobox 1b [Source:ZFIN;Acc:ZDB-GENE-070424-11]	gene expression - steroid metabolic process - neurogenesis - immune system process - sex differentiation - transcription factor activity -
msi1	OT43	Musashi RNA Binding Protein 1	nervous system development - mRNA surveillance pathway

Non-preference DE genes

fam135b	OT5	Family With Sequence Similarity 135 Member B	hydrolase activity
adcyap1r1	OT5	Anaphase Promoting Complex Subunit 11	response to stimulus - response to estradiol - reproduction - primary metabolic process - purine nucleotide metabolic process - cAMP biosynthetic process - calcium ion transport - regulation of hydrolase activity - reproductive process - sexual reproduction - regulation of response to stimulus - signal transduction - G-protein coupled receptor signaling pathway - cAMP signaling pathway - Neuroactive ligand-receptor interaction
slco3a1	OT5	Solute Carrier Organic Anion Transporter Family Member 3A1	ion transport - prostaglandin transport - transporter activity
sv2a	OT5	synaptic vesicle glycoprotein 2A [Source:HGNC Symbol;Acc:HGNC:20566]	localization - ion transport - neurotransmitter transport - ECM-receptor interaction
tmem151b	OT5	(Transmembrane Protein 151B)	N/A
spock3	OT5	SPARC/Osteonectin, Cwcv And Kazal Like Domains Proteoglycan 3)	primary metabolic process - regulation of hydrolase activity - localization - response to stimulus - signal transduction - ion binding
wbp1	OT5	WW domain binding protein 1 [Source:ZFIN;Acc:ZDB-GENE-040426-1830]	protein binding
slc7a2	OT8	solute carrier family 7 (cationic amino acid transporter, y+ system), member 2 [Source:ZFIN;Acc:ZDB-GENE-041212-5]	primary metabolic process - response to external stimulus - localization - ion transport - ion transmembrane transport
rpl13	OT9	Ribosomal Protein L13	localization - SRP-dependent cotranslational protein targeting to membrane - gene expression - translation - Ribosome pathway
rpl14	OT9	Ribosomal Protein L14	protein localization - gene expression - SRP-dependent cotranslational protein targeting to membrane - Ribosome pathway
rs12	OT9	ribosomal protein S12	Ribosome pathway
prkg1	OT14	Protein Kinase, CGMP-Dependent, Type I	regulation of hydrolase activity - response to external stimulus - neurogenesis - signal transduction - cGMP-mediated signaling - ion channel regulator activity - abnormality of the eye - Long-term depression
dnajc5	OT16	DnaJ Heat Shock Protein Family (Hsp40) Member C5	localization - neurotransmitter transport - synaptic vesicle localization - synaptic signaling - signal release from synapse
flot1	OT16	flotillin 1b [Source:ZFIN;Acc:ZDB-GENE-020430-2]	synaptic signaling - neurogenesis - response to stimulus - localization - signal transduction - Ras protein signal transduction - Rho protein signal transduction - glutamate receptor binding
myrf	OT16	(Myelin Regulatory Factor	gene expression - nervous system process - neurogenesis - transcription factor activity - hydrolase activity
sgk3	OT16	serum/glucocorticoid regulated kinase family, member 3 [Source:ZFIN;Acc:ZDB-GENE-070424-62]	localization - ion transmembrane transport - response to stimulus - signal transduction - ion channel regulator activity
stxbp6	OT16	Syntaxin Binding Protein 6)	localization - regulation of transport - ion binding - Ras GTPase binding - Rho GTPase binding
gjb1	OT31	Gap Junction Protein Beta 1	localization - transmembrane transport - reproductive system development - reproductive process - transmembrane transporter activity - channel activity - Behavioral abnormality - Sensory impairment - abnormality of the eye
plp1	OT31	Myelin proteolipid protein	gene expression - neurogenesis - response to stimulus - synaptic signaling - signal transduction - Sensory neuropathy - abnormality of the nervous system - Cognitive impairment - abnormality of the optic nerve
rhoab	OT31	ras homolog gene family, member Ab [Source:ZFIN;Acc:ZDB-GENE-040322-2]	localization - response to stimulus - signal transduction - Ras protein signal transduction - Rho protein signal transduction - hydrolase activity - Wnt signaling pathway

bcas1	OT32	breast carcinoma amplified sequence 1	N/A
cd59	OT32	CD59 molecule, complement regulatory protein [Source:ZFIN;Acc:ZDB-GENE-030131-7871]	gene expression - response to external stimulus - primary metabolic process - signal transduction
mbp-like	OT32	loc103478274 - myelin basic protein-like [Poecilia reticulata (guppy)]	N/A
mag	OT32	myelin associated glycoprotein [Source:ZFIN;Acc:ZDB-GENE-041217-24]	response to external stimulus - neurogenesis - regulation of neurological system process - ion binding - abnormality of the eye - abnormality of the optic nerve - abnormality of vision - Cognitive impairment
paqr6	OT32	progestin and adipoQ receptor family member VI [Source:ZFIN;Acc:ZDB-GENE-090714-1]	response to stimulus - signal transduction - hormone-mediated signaling pathway - signal transducer activity - steroid hormone receptor activity
ppp1r14a	OT32	Protein Phosphatase 1 Regulatory Inhibitor Subunit 14A	response to stimulus
slc2a3	OT34	Solute Carrier Family 2 Member 3	localization - transmembrane transport - response to stimulus - transporter activity - Cognitive impairment - Behavioral abnormality
cxorf57	OT40	Chromosome X Open Reading Frame 57	protein binding
smg8	OT40	SMG8 nonsense mediated mRNA decay factor [Source:ZFIN;Acc:ZDB-GENE-091204-279]	primary metabolic process - regulation of gene expression
aars2	OT40	Alanyl-TRNA Synthetase 2, Mitochondrial	primary metabolic process - gene expression - translation - ion binding - Cognitive impairment - Behavioral abnormality - Abnormal nervous system electrophysiology

TELENCEPHALON

Preference DE genes

c2cd5	T13	C2 calcium dependent domain containing 5 [Source:HGNC Symbol;Acc:HGNC:29062]	response to stimulus - localization - transmembrane transport - signal transduction - transmembrane receptor protein tyrosine kinase signaling pathway - ion binding
dhx36	T13	DEAH-box helicase 36 [Source:HGNC Symbol;Acc:HGNC:14410]	localization - response to external stimulus - gene expression - regulation of transport - ion binding - hydrolase activity
rtbs	T13	Putative RNA-directed DNA polymerase from transposon BS	N/A
fcrla	T4	Fc receptor like A [Source:HGNC Symbol;Acc:HGNC:18504]	cell differentiation - protein binding
trinity_dn171917_c2_g2	T4	N/A	N/A
rps2	T46	Ribosomal Protein S2	response to stimulus - primary metabolic process - translation - Ribosome pathway
rpl35a	T37	Ribosomal Protein L35a	gene expression - translation - RNA binding - Ribosome pathway
rpl18	T37	Ribosomal Protein L18	gene expression - translation - RNA binding - Ribosome pathway
eef2	T37	Eukaryotic Translation Elongation Factor 2	response to external stimulus - response to estradiol - gene expression - translation - primary metabolic process - hydrolase activity - RNA binding - ion binding - abnormality of the eye - AMPK signaling pathway - oxytocin signaling pathway - abnormality of the nervous system
rpl4	T37	60S ribosomal protein L4-A	localization - SRP-dependent cotranslational protein targeting to membrane - protein localization - gene expression - translation - Ribosome pathway - RNA binding
ndufb9	T37	NADH:Ubiquinone Oxidoreductase Subunit B9	primary metabolic process - NADH dehydrogenase activity - sensory perception - abnormality of higher mental function - abnormality of the eye - Visual impairment - abnormality of the optic nerve - Morphological abnormality of the central nervous system - Parkinson's disease - Huntington's disease - Alzheimer's disease
rps17	T37	Ribosomal Protein S17	immune system process - localization - primary metabolic process - RNA processing - translation - RNA binding - Ribosome pathway -abnormality of the nervous system

Non-preference DE genes

ttc39b	T13	Tetratricopeptide Repeat Domain 39B	N/A
plk2	T31	polo like kinase 2 [Source:HGNC Symbol;Acc:HGNC:19699]	primary metabolic process - behavior - response to stimulus - cognition - learning or memory - neurogenesis - signal transduction - Ras protein signal transduction - ion binding - FoxO signaling pathway

adgrb1	T29	adhesion G protein-coupled receptor B1 [Source:HGNC Symbol;Acc:HGNC:943]	localization - transport - nervous system development - neurogenesis - response to stimulus - synaptic signaling - synapse organization - signal transduction - ion binding - G-protein coupled receptor activity - signaling receptor activity
jun	T20	Jun proto-oncogene, AP-1 transcription factor subunit [Source:HGNC Symbol;Acc:HGNC:6204]	behavior - response to external stimulus - cognition - learning or memory - localization - sensory organ development - regulation of hydrolase activity - response to cAMP - signal transduction - MAPK cascade - cAMP response element binding - Pathways in cancer - Oxytocin signaling pathway - cAMP signaling pathway - Estrogen signaling pathway - Wnt signaling pathway - GnRH signaling pathway - MAPK signaling pathway - Neurotrophin signaling pathway
rtn4rl2	T23	Reticulon 4 Receptor Like 2	response to stimulus - neurogenesis - signal transduction - STAT cascade - receptor activity

[δ] Compiled from g:Profiler[2]

Table S2: Chromosome enrichment.

		Preference DE genes		Non-preference DE genes	
		OPTIC TECTUM	TELENCEPHALON	OPTIC TECTUM	TELENCEPHALON
Autosomes	LG1	13	4	1	2
	LG2	9	7	5	0
	LG3	4	5	2	0
	LG4	7	8	1	2
	LG5	9	3	3	4
	LG6	10	5	2	1
	LG7	8	4	2	0
	LG8	11	6	3	1
	LG9	6	5	1	2
	LG10	6	1	1	0
	LG11	8	4	3	1
	LG13	4	9 (p=0.03)	0	0
	LG14	7	0	3	0
	LG15	3	1	3	1 (p=0.03)
	LG16	12	11 (p=0.01)	5 (p=0.02)	1
	LG17	4	7	3	1
	LG18	8	0	2	1
	LG19	10	6	1	1
	LG20	8	4	1	0
	LG21	11 (p=0.05)	2	1	0
	LG22	9	2	1	2
	LG23	3	2	5	0
X chromosomes	LG12	10	0	1	1

Numbers correspond to number of Preference DE genes in each chromosome. Remaining genes map to unplaced scaffolds. P-values correspond to one-tail fisher’s exact test for gene enrichment in each chromosome considering the number of total genes mapped to each linkage group. Non-significant P-values are > 0.05.

Table S3: Co-expression network properties.

	OPTIC TECTUM	TELENCEPHALON
Total number of transcripts	20396	19571
Transcripts with correlation >|0.4|	6297	3540
Preference DE genes remaining in network	57	12
Non-preference DE genes remaining in network	31	6
ATTRIBUTES		
Network average degree (se)	6.7 (3.9)	10.8 (5.5)
Network average Clustering Coefficient (se)	0.2 (0.6)	0.3 (0.6)
Network average Neighborhood Connectivity (se)	10.8 (3.2)	19.0 (4.6)
MODULES		
Number of modules identified before merging	92	160
Number of modules identified before merging	43	89

Table S4: List of know social behavior/ mate preference genes used in this study. Compiled from[4-7].

Social behavior / mate preference gene	Social behavior subcategory
acsl1	General social
acsl1b	General social
acsl3	General social
acsl3b	General social
adra1b	Receptivity
adrb1	Receptivity
cant1 (apyrase)	General social
cyp191b (brain aromatase)	Receptivity
c14sr	General social
celsr1	General social
dhps	General social
gnrh2	General social
neui (isotocin)	General social
itgb5	General social
kpnb1	General social
napb	General social
napa	General social
nlgn2	Mate Preference
nlgn3 (neuroligin-3)	Mate Preference
serpin1 (neuroserpin)	Mate Preference
nicn1	General social
grin1b (nmdarb)	Mate Preference
grin1 (nmdar)	Mate Preference
plat (tpa)	Mate Preference
sf3b1	General social
sg10	Mate Preference
stmn2	Mate Preference
stmn2par	General social
th	Receptivity
avt (vasotocin)	General social
avpi	General social
avpr2	General social
egr1	Mate Preference
egr1a	Mate Preference
egr1b	Mate Preference
egr3	General social
bdnf	General social
neuroD	General social
npas4	General social
wnt3	General social

Table S5: List of know Synaptic Plasticity genes (SPG) and Immediate Early genes (IEG) used in this study. Compiled from[3].

IEG	Late Response Genes	Long Term Potentiation (LTP)	Long Term Depression	Cell Adhesion	Extracellular Matrix & Proteolytic Processing	CREB Cofactors	Neuronal Receptors	Postsynaptic Density
arc	*inhba*	*adcy1*	*gnai1*	*adam10*	*adam10*	*akt1*	*ephb2*	*adam10*
bdnf	*synpo*	*adcy8*	*gria1*	*cdh2*	*mmp9*	*camk2g*	*gabra5*	*arc*
cebpb		*bdnf*	*gria2*	*grin2a*	*plat*	*grin1*	*gria1*	*dlg4*
cebpd		*camk2a*	*gria3*	*grin2b*	*tpa*	*grin2a*	*gria2*	*gria1*
creb1		*camk2g*	*gria4*	*ncam1*	*reln*	*grin2b*	*gria3*	*gria3*
crem		*cdh2 (n-cadherin)*	*grip1*	*pcdh8*	*timp1*	*grin2c*	*gria4*	*gria4*
egr1		*cnr1*	*grm1*	*ppp2ca*		*grin2d*	*grin1*	*grin1*
egr2		*gabra5*	*grm2*	*reln*		*mapk1*	*grin2a*	*grin2a*
egr3		*gnai1*	*igf1*	*tnf*		*ppp1ca*	*grin2b*	*grin2b*
egr4		*gria1*	*mapk1*	*cdh2*		*ppp1cc*	*grin2c*	*grin2c*
fos		*gria2*	*nos1*			*erk2*	*grin2d*	*grm1*
homer1		*grin1*	*ngfr*				*grm1*	*grm3*
jun		*grin2a*	*pick1*				*grm2*	*homer1*
junb		*grin2b*	*plat (tpa)*				*grm3*	*pick1*
klf10		*grin2c*	*ppp1ca*				*grm4*	*synpo*
mmp9		*grin2d*	*ppp1cc*				*grm5*	*psd95*
nfkb1		*mapk1*	*ppp1r14a*				*grm7*	
nfkbib		*mmp9 (gelatinaseb)*	*ppp2ca*				*grm8*	
ngf		*ntf4*	*ppp3ca*				*ntrk2*	
nptx2		*ntrk2*	*prkca*					
nr4a1		*plcg1*	*prkg1*					
ntf3		*ppp1ca*	*cpi-17*					
pcdh8		*ppp1cc*						
pim1		*ppp3ca*						
plat		*prkca*						
rela		*prkcg*						
rgs2		*rab3a*						
rheb		*ywhaq*						
srf								
tnf								
trip9								

Table S6: Go enrichment for modules of interest.

MODULE	Module size	Preference DE genes*	Non-preference DE genes*	Social - mating*	SPG/IEG*	Relevant GO enriched terms (statistically significant enrichment with p<0.1)
OPTIC TECTUM						
OT5	863				⬛	transmembrane signaling receptor activity - potassium channel activity -voltage-gated channel activity - receptor tyrosine kinase binding - signaling receptor activity
OT9	273	🟥				Multiple DNA binding terms - translation initiation factor binding - thyrotropin-releasing hormone receptor binding - cotranslational protein targeting to membrane - aromatic compound catabolic process
OT12	247	🟥				AMPA glutamate receptor activity - protein homodimerization activity - androgen receptor activity - protein homodimerization activity - regulation of neuron projection development
OT15	13	🟥				sterol transporter activity - sterol desaturase activity -negative regulation of protein tyrosine kinase activity - NFAT protein import into nucleus
OT16	258		▨			hydrolase activity - prostaglandin-E synthase activity - prostaglandin-E synthase activity - Rho GTPase binding - forebrain astrocyte differentiation - peptide transport - response to stimulus - myosin binding
OT17	659			🟩		G-protein coupled receptor activity - ionotropic glutamate receptor binding - protein tyrosine kinase binding - calcium channel regulator activity - glutamate binding - G-protein coupled glutamate receptor activity
OT21	45	🟥				Sodium/neurotransmitter: amino acid symporter activity - Rho GDP-dissociation inhibitor binding - neurotransmitter transporter activity - Various G-protein coupled related terms - myosin V binding - pheromone-dependent signal transduction - steroid sulfotransferase activity - hormone activity
OT24	388	🟥				glutamate receptor activity - ligand-gated calcium channel activity - voltage-gated calcium channel activity - lipoprotein transporter activity
OT31	78		▨			canonical Wnt signaling pathway - pheromone-dependent signal transduction - neurotransmitter: sodium symporter activity- Rho GDP-dissociation inhibitor binding
OT32	66		▨			Wnt-activated receptor activity - L-tyrosine transmembrane transporter activity - aromatase activity - regulation of Notch signaling pathway - auditory receptor cell fate commitment
OT40	711					translation initiation factor activity - transcription factor binding - purine-nucleoside phosphorylase activity
TELENCEPHALON						
T2	7				⬛	transcription factor activity - positive regulation of mating-type specific transcription from RNA polymerase II promoter - positive regulation of cellular amine catabolic process -
T4	113	🟥				sodium-dependent organic cation transport - cystine:glutamate antiporter activity - neurotransmitter transporter activity - prostaglandin F receptor activity - cation:cation antiporter activity
T12	18			🟩	⬛	protein tyrosine phosphatase activity - transcription factor activity - neuregulin receptor activity - voltage-gated calcium channel activity - ERK1 and ERK2 cascade - negative regulation of kinase activity
T13	1642				⬛	Neurogenesis - signal transduction - synaptic signaling - modulation of synaptic transmission - modulation of signaling - response to stimulus
T23	10		▨			methionine-tRNA ligase activity - neuregulin receptor activity - purine nucleotide binding - inter-male aggressive behavior - response to follicle-stimulating hormone - response to gonadotropin - regulation of synapse maturation
T29	13		▨			sodium:potassium:chloride symporter activity - kinase binding - hormone receptor binding - Ras guanyl-nucleotide exchange factor activity - synaptic vesicle coating - motor learning

T31	2		▨			signal transducer activity - transmembrane receptor protein tyrosine kinase activity - transferase activity - gonadotropin-releasing hormone (GnRH) - neuronal migration to the hypothalamus - neuropilin signaling pathway
T32	28				⬛	modulation of synaptic transmission - motor behavior - regulation of neurotransmitter transport - glutamate receptor signaling pathway - synaptic transmission, glutamatergic - glutamate receptor activity - receptor activity - AMPA glutamate receptor activity - signaling receptor activity
T37	982	🟥				hydrolase activity - sterol O-acyltransferase activity - amide transmembrane transporter activity - structural constituent of ribosome - protein channel activity - peptide transmembrane transporter activity
43	72			🟩	⬛	learning or memory - behavior - cognition - signal transduction - translation repressor activity - neurotrophin receptor activity - purine nucleoside binding - sterol-transporting ATPase activity - glutamate binding - synapse organization - signal release from synapse
46	29	🟥				pyruvate dehydrogenase activity - ribosome binding - laminin receptor activity - nuclear-transcribed mRNA catabolic process

∗Enrichment of modules in each GO category was determined using a p-value threshold of 0.1. Color squares indicate the gene enrichment for each module: modules enriched in Preference DE genes have a red square, non-preference DE genes a dark grey square, known preference/social behavior genes a green square and synaptic plasticity genes & immediate early genes a black square. Module size corresponds to number of genes within a module after filtering out genes without genes correlations >0.4.

Table S7: Pathways for Preference DE genes.

OPTIC TECTUM									TELENCEPHALON		
Preference DE Genes Pathways	Module OT5	Module OT9	Module OT12	Module OT15	Module OT17	Module OT21	Module OT24	Module OT40	Preference DE Genes Pathways	Module T13	Module T37
Ras signaling pathway	■	■							*Translocation of GLUT4 to the plasma membrane*	■	
Rap1 signaling	■								*Toll-Like Receptors Cascades*	■	
Cocaine addiction	■								*Parkinson's disease*		■
Phototransduction	■								*Huntington's disease*		■
Sodium/Calcium exchangers	■								*Alzheimer's disease*		■
Ribososme		■							*Oxytocin signaling pathway*		■
Notch signaling pathway		■							*AMPK signaling pathway*		■
Signaling by Wnt		■						■	*Ribosome*		■
MAPK family signaling cascades		■							*Translation*		■
NOD-like receptor signaling pathway			■								
Membrane Trafficking				■							
Vesicle-mediated transport				■							
Purine metabolism					■						
Oxytocin signaling pathway					■						
cGMP-PKG signaling pathway					■						
Signal Transduction		■			■						
Gastrin-CREB signaling pathway via PKC and MAPK					■						
Protein folding						■					
Glutamatergic synapse							■				
Dopaminergic synapse							■				
cAMP signaling pathway							■				
Long-term depression							■				
Taste transduction							■				
Phase 0 - rapid depolarisation							■				
TCF dependent signaling in response to WNT								■			
Neurotransmitter Release Cycle								■			

Table S8: Pathways for Social DE genes.

OPTIC TECTUM Social DE genes Pathways	TELENCEPHALON Social DE genes Pathways
Activation of NMDA receptor upon glutamate binding and postsynaptic events	*Activated TLR4 signaling*
AGE-RAGE signaling pathway in diabetic complications	*MHC class II antigen presentation*
Alzheimer's disease	*SRP-dependent cotranslational protein targeting to membrane*
Calcium signaling pathway	*Toll-Like Receptors Cascades*
cAMP signaling pathway	*Diseases of signal transduction*
Cocaine addiction	*Signaling by Activin*
Dopaminergic synapse	*Translation*
Glutamate Binding, Activation of AMPA Receptors and Synaptic Plasticity	*Gene Expression*
Glutamatergic synapse	*Signal Transduction*
GnRH signaling pathway	*Transmembrane transport of small molecules*
Huntington's disease	*Activation of HOX genes during differentiation*
JNK (c-Jun kinases) phosphorylation and activation mediated by activated human TAK1	*Apoptotic factor-mediated response*
Long-term depression	*Glycoprotein hormones*
Long-term potentiation	*Ligand-independent caspase activation via DCC*
MAPK targets/ Nuclear events mediated by MAP kinases	*NOTCH2 intracellular domain regulates transcription*
NADPH regeneration	*Stimuli-sensing channels*
Neuroactive ligand-receptor interaction	*Transcriptional Regulation by TP53*
Neurotrophin signaling pathway	*Transmission across Electrical Synapses*
NOD-like receptor signaling pathway	
Progesterone-mediated oocyte maturation	
Rap1 signaling pathway	
Retrograde endocannabinoid signaling	
Serotonergic synapse	
Signal attenuation	
Signal Transduction	
Signal transduction by L1	
Signaling by Rho GTPases	
Stimuli-sensing channels	
Regulation of actomyosin structure organization	
Wnt signaling pathway	

Table S9: Social DE genes transcription factor motifs represented among Preference DE genes.

Social DE genes TF motif	Biological role in behavior	Preference DE genes with predicted TF binding site	Pattern in attractive relative to dull treatment$^{\delta}$	
			Preference	Non preference
		OPTIC TECTUM		
otx1	Neuronal differentiation associate with social challenges[8,9] - autism[10]	*pdzrn3, thap1, txnrd2, rgl2*	DOWN	UP
zic4	-	*tubb4a, pdzrn3, mrps2, thap1, rnf165, npr2, tbc1d10b, tmem198, far1, rgl2*	DOWN	DOWN
pou3f4	Social challenges[8] autism-like behavior[11]	*gca, gria3, scn2a, nlrp3, zic4, scn8a, far1*	DOWN	DOWN
zic1	Involved in neurogenesis and behavioral abnormalities in mice[12]	*tac1, tubb4a, cand1, gca, pdzrn3, mrps2, gria3, rnf165, mrpl9, tpm3, npr2, ablim2, tbc1d10b, zic4, psmd2, txnrd2, tmem198, scn8a, far1, rgl2*	DOWN	DOWN
pbx1	neuroendocrine stress signaling - social or fear inducing behavior[13]	*gca, nlrp3, ablim2, zic4, txnrd2, far1, tac1, scn2a, mrpl9, scn8a*	DOWN	UP
htf4		*tac1, tubb4a, dtx4, cand1, gca, mrps2, gria3, mrpl9, tpm3, npr2, nlrp3, ablim2, ddb1, tbc1d10b, psmd2, txnrd2, tmem198, scn8a*	DOWN	UP
satb1	Alters temporal expression of multiple IEG and SPG controlling the genomic response at the basis of complex behaviors, learning and memory[14]	*tubb4a, dtx4, cand1, gca, pdzrn3, mrps2, gria3, thap1, scn2a, rnf165, mrpl9, tpm3, npr2, nlrp3, ablim2, ddb1, tbc1d10b, zic4, psmd2, txnrd2, tmem198, scn8a, far1, rgl2*	UP	DOWN
sall1	Townes-Brocks syndrome[15]	*dtx4, tubb4a, cand1, gca, pdzrn3, mrps2, tpm3, npr2, ablim2, tbc1d10b, tmem198, rgl2*	DOWN	DOWN
znf718	-	*gca, mrpl9*	UP	DOWN
foxk2	Cognitive deficits with age and Parkinson's disease[16,17]	*tac1, cand1, pdzrn3, mrps2, scn2a, rnf165, mrpl9, nlrp3, ddb1, far1*	DOWN	UP
klf5	Family of co-regulators and integrators of steroid hormone actions - Social challenges[8]	*tubb4a, dtx4, cand1, pdzrn3, mrps2, gria3, thap1, rnf165, mrpl9, tpm3, npr2, ablim2, ddb1, zic4, txnrd2, tmem198, scn8a, far1, rgl2*	DOWN	UP
ap2	Gene expression regulation in synaptic plasticity associated with social challenges (	*tubb4a, dtx4, pdzrn3, mrps2, thap1, scn2a, rnf165, mrpl9, npr2, ablim2, ddb1, tbc1d10b, psmd2, txnrd2, tmem198, scn8a, far1*	DOWN	DOWN
gata2	Social challenges[8], social eavesdropping[18]	*tac1, tubb4a, dtx4, cand1, gca, gria3, thap1, scn2a, rnf165, mrpl9, nlrp3, ddb1, tmem198, scn8a, npr2, pdzrn3, ablim2, txnrd2, tpm3, psmd2*	UP	-
		TELENCEPHALON		
neurod2	Regulation of neurogenesis, social behavior (	*rpl18, c2cd5, ndufb9, dhx36, rps17, rps2, ndufb9, rpl35a, eef2*	UP	DOWN
egr2	Neuronal plasticity -reproduction or social behavior - Social challenges[8], autism[19] memory and learning[20]	*rpl4, rpl18, fcrla, rps2, c2cd5, ndufb9, eef2, rps17, dhx36*	UP	UP
Klf7	Family of co-regulators and integrators of steroid hormone actions, autism and learning disorders[21], learning[22]	*rps2, ndufb9, eef2, rpl18, c2cd5, rpl4, rps17, rpl35a*	DOWN	UP
satb1	Alters temporal expression of multiple IEG and SPG controlling the genomic response at the basis of complex behaviors, learning and memory[14]	*rpl18, c2cd5, rps2, eef2, rpl4, dhx36, rpl35a, rps17, fcrla, ndufb9*	UP	UP

$^{\delta}$Whether transcription factors are down-regulated or up-regulated in females exposed to an attractive vs dull male. Details on expression patterns for each transcription factor can be found in Fig. S7

Table S10: Assembly statistics.

	De novo assembly	Genome-guided assembly	Reference Transcriptome
Number of transcripts:			
before filtering	704766	49012	
after ncRNA filter	703564	48901	61092
After best isoform selection	490912	26138	
After ORF filering	44769	-	
Average length	895	2730	1450
Maximum length	18612	93950	93950
Minimun length	297	67	155
N50	1380	3599	3046
N90	351	1477	411
Merging statistics			
De novo assembly transcripts	-	-	25792
Average length	-	-	2756
Genome-guided assembly transcripts	-	-	35300
Average length	-	-	496
BLAST & Annotation statistics			
Transcripts with >1 Blast hit			17517
Transcripts with no Blast hits			3681
GO annotations			17142

Table S11: Samples.

		Sample number	
LINE	TREATMENT	Optic Tectum	Telencephalon
PREFERENCE LINES	Attractive male	25* (5)	30 (6)
	Dull Male	30 (6)	30 (6)
	Female	25* (5)	30 (6)
NON-PREFERENCE LINES	Attractive male	15 (3)	15 (3)
	Dull Male	15 (3)	15 (3)
	Female	15 (3)	10* (2)

Samples were arranged in non-overlapping pools of 5 individuals for RNA-Seq. Number of female brains, and corresponding pools in parenthesis, used for each treatment in Preference and Non-preference lines. (*) One pool excluded from this treatment as a statistical outlier.

CAPTIONS FOR DATABASES

Additional Data table S1 (separate file)

Optic tectum normalized count data for differentially expressed genes

Additional Data table S2 (separate file)

Databases S2: Telencephalon normalized count data for differentially expressed genes

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