DIFFERENT MATING CONTEXTS LEAD TO EXTENSIVE REWIRING OF FEMALE BRAIN COEXPRESSION NETWORKS IN THE GUPPY

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ABSTRACT

Understanding the basis of behavior requires dissecting the complex waves of gene expression that underlie how the brain processes stimuli and produces an appropriate response. In order to determine the dynamic nature of the neurogenomic network underlying mate choice, we use transcriptome sequencing to capture the female neurogenomic response in two brain regions involved in sensory processing and decision-making under different mating and social contexts. We use differential coexpression (DC) analysis to evaluate how gene networks in the brain are rewired when a female evaluates attractive and non-attractive males, greatly extending current single-gene approaches to assess changes in the broader gene regulatory network. We find the brain experiences a remarkable amount of network rewiring in the different mating and social contexts we tested. Further analysis indicates the network differences across contexts are associated with behaviorally relevant functions and pathways, particularly learning, memory and other cognitive functions. Finally, we identify the loci that display social context-dependent connections, revealing the basis of how relevant neurological and metabolic pathways are differentially recruited in distinct social contexts. More broadly, our findings contribute to our understanding of the genetics of mating and social behavior by identifying gene drivers behind behavioral neural processes, illustrating the utility of DC analysis in neurosciences and behavior.

INTRODUCTION

Understanding how behaviors are encoded within the genome has been a long-standing and contentious question. Answering it requires determining how genes and gene expression are connected to behavior. In evolutionary biology, social and mating behaviors are of particular interest due to their central role in sexual selection and other core evolutionary processes. Studying differences in gene sequence and/or gene expression associated with different phenotypes and treatments has greatly contributed to our understanding of the genes encoding many behavioral traits, such as aggressive behavior [1,2], burrowing behavior [3], nurturing behavior[4], response to song [5,6], response to intruders [7-9].

In order to understand the molecular mechanisms behind the control of behavior, we need to determine how neurogenomic responses, rapid cascades of gene expression changes in the brain [10], are related to different contexts and stimuli, and the behavioral responses they modulate [9,11-14]. Gene coexpression networks are a powerful method to analyze and visualize complex and large transcriptional datasets, providing a tool to understand the multidimensional nature of gene networks [15,16]. Coexpression networks are constructed based on correlations between expression levels of thousands of genes across different conditions, revealing clusters, or modules, of co-regulated genes with similar transcription profiles [11,12,15]. Genes that cluster together within coexpression networks have been shown to be part of the same regulatory pathways and/or possess similar biological functions [17-19], making these networks a particularly useful approach in non-model organisms for which knowledge of gene function may be limited. Coexpression networks have contributed to our understanding of the modular structure of brain transcriptional changes associated with many behaviors in diverse organisms, from honey bees to humans [7,11,12,20-23].

Capturing and understanding the plasticity that characterizes the neurogenomic responses that mediate behavior[24] requires going beyond approaches that only allow us to visualize gene

networks. This is effectively done by quantifying how the connections and modularity of gene networks change under different conditions, i.e. how the network is rewired. Differential network analysis methods capture the dynamic nature of transcriptional responses, providing details on which modules of the network are most affected, and condition-specific changes in regulatory relationships between genes [17,25,26]. Therefore, differential network analysis can be a powerful approach to identify genes with key regulatory roles. Differential network analysis has been previously used in cancer and disease biology to identify regulatory pathways at the basis of many diseases [26-32]. The power of differential network approaches can be particularly useful in studies of behavior, which is often controlled by rapid waves of gene expression changes instead of changes in a handful of genes. Despite their potential, these approaches have rarely been applied to behavior research [20,29].

Here, we focus on mating behavior in the Trinidadian guppy (*Poecilia reticulata*), a classic system for the study of mating behavior due to the repeated coevolution of female preference and male color across populations [33-36]. We used laboratory wild-type and selection lines with strong and proven female preference for colorful males, recapitulating the female preference seen in wild populations [11,37]. We compare the neurogenomic response across contrasting mating and social contexts by exposing females to i) a colorful (attractive) male that matches their preference, ii) to a dull (non-attractive) male or iii) another female in a general social interaction. We used previously published RNA-seq data for the optic tectum, a brain region involved in sensory processing of visual signals, and the telencephalon, the region responsible for integrating those signals and mediating complex decision-making, including social and mating decisions [38-40].

We previously identified genes that are differentially expressed in response to these behavioral treatments[11]. Our goal here, was to transcend differential expression analysis of each gene as an independent unit, by implementing differential network analysis to identify gene interactions that change in response to mating/social context. We use a series of analyses to estimate how much

coexpression networks change across social contexts, and to identify the biological pathways within the networks that are being differentially recruited across these social contexts, by determining which specific parts of the coexpression network undergo significant rewiring in the brain. We found that female brain coexpression networks undergo remarkable rewiring in response to mating and social context, with extraordinary flexibility associated with the evaluation of males with different levels of attractiveness. Interestingly, brain expression profiles are much less preserved across social contexts within one species, than between related species in the absence of social interactions[41]. We also identified specific modules and gene pairs associated with cognition, learning and memory which are flexibly utilized between networks. Moreover, these gene pairs can represent subnetworks/groups of genes that are differentially recruited according to the mating/social context.

METHODS

Study system and behavioral trials: We carried out behavioral trials with laboratory populations in which females have clear preference for colorful males. We used laboratory guppies derived from wild-type Trinidad populations and replicate laboratory selection lines selected for larger relative brain size [42]. We focused on those guppy lines (wild-type and three separate brain-size selection lines) that share a clear preferences for colorful males [37], thus ensuring any patterns we detect are due to the neurogenomic processes associated with the studied social contexts. Moreover, we have recently shown that this preference is associated with a unique neurogenomic signature [11]. Six month-old virgin females from both the wild-type and large-brain females were exposed to one of three experimental conditions: females were exposed to an attractive male, a dull male, or another female, the latter representing a control general social interaction. We created non-overlapping pools from five individual females for each brain region in order to have sufficient RNA for sequencing, for a total of three pools per treatment per line. These 18 pools per brain region

encompass variation from 18x5 = 90 females. We allowed each focal female to observe the presented fish for 10 minutes before ending the experiment and dissecting the brain.

Sequencing and assembly construction: We used RNAseq to quantify gene expression levels in two components of the brain, the optic tectum, associated with sensory processing of visual signals, and the telencephalon, responsible for decision-making, after exposure to each of the behavioral treatments. We had three replicate non-overlapping pools of five females each for each treatment and each tissue, see SI for more details. Samples were sequenced across 10 lanes on an Illumina HiSeq 4000 yielding on average 52 million 75bp paired-end reads per sample. Samples were filtered and trimmed based on quality before constructing a hybrid non-redundant reference transcriptome assembly (RefTrans) by merging a genome-guided assembly and a *de novo* assembly with the totality of the reads obtained as previously described [11] and in the SI methods. We then quantified gene expression by mapping paired reads for each sample separately to the Reference Transcriptome. After read mapping and filtering a total of 20,396 transcripts in the optic tectum and 19,571 in the telencephalon were maintained for all downstream analysis.

Coexpression networks: We built gene coexpression networks with normalized read counts for each treatment in the optic tectum and telencephalon (n=18 for each tissue and n=12 for each pairwise comparison) using the *Weighted gene correlation network analysis*, WGCNA, package in R[15,43] (See SI methods for additional details). This package allows us to build gene networks based on the pairwise correlations between gene expression values across treatments. Figs. S1C and S2C show details of the numbers of transcripts included in each coexpression network after filtering for genes with less than median levels of variance across all samples[15,43]. We then used the Dynamic Tree Cut method as implemented in WGCNA to detect gene modules within each network[44]. We further merged modules with highly correlated expression values by estimating module eigengenes as described in [15,43] (Fig. S4).

Measures of network preservation: We conducted a differential analysis of eigengene networks to evaluate overall preservation between treatment coexpression networks as in Langfelder & Horvath, (2007) [45]. This procedure calculates an eigengene preservation measure for each module $C_{mod}(Preserv^{treat1, treat2})$ relative to consensus modules, which can be averaged to estimate $D_{Preserv}$ between the two networks. $D_{Preserv}$ reflects the overall preservation between two networks, where higher values indicate a stronger preservation of correlation values between pairs of eigengenes in the two compared networks (see Equation 6 in [45]). We evaluated whether our results were robust to the parameter combination used to estimate $D_{Preserv}$ by calculating this measure over multiple parameter combinations. More specifically, we changed the merging height (from 0.25 to 0.4), the clustering method (*tree* vs *hybrid*) and hybrid tree cut height (from 0.98 to 0.999; Tables S2, S3 and S4). In all cases we report an average of $D_{Preserv}$ calculated over multiple combinations of module identification parameters.

Module preservation: To evaluate network preservation in more detail, we used multiple measures of module preservation introduced by Langfelder et al. (2011) [46] to identify modules of interest that differ in gene connectivity between behavioral treatments. We focused on composite measures of module preservation, which aggregate multiple preservation statistics and offer a general measure of module preservation [46]. We contrasted modules between networks following Oldham et al. (2006) [41], using the Attractive treatment network as reference. We initially estimated $Z_{summary}$ for all modules, using reported thresholds in [46] to identify non-preserved modules between networks as detailed in the SI methods. Because $Z_{summary}$ is sensitive to module size we also used the *medianRank* to identify non-preserved modules, which is less sensitive to module size. A module was considered to be be non-preserved between networks if it had low values of $Z_{summary}$ AND high values of *medianRank* (using 200 permutations).

Differentially coexpression analysis: We used *Bayes approach for Differential Coexpression Analysis* (BFDCA) [47], which identifies pairs of genes that have different correlation patterns in two

conditions [27,48-52]. BFDCA is based on WGCNA and was shown to have very good performance compared to previous methods in accuracy and robustness using both simulation and experimental data [47]. This untargeted approach to differential coexpression (DC) analysis uses a combined Bayes factor, a ratio of marginal likelihood of the data between the two alternative hypotheses, to evaluate which genes are differentially correlated in two conditions [47]. After identifying DC gene pairs with BFDCA, we controlled for false positives and accounted for multiple testing, by integrating a random permutations approach [53]. Using 1000 permuted datasets, a DC gene pair was considered significant if the Bayes factor for the actual expression data was larger than the 1% tail of the permutated data Bayes factor distribution.

RESULTS

Low coexpression network preservation across mating contexts

We built coexpression networks by treatment for each brain tissue separately using weighted gene coexpression network analysis (WGCNA)[15]. The methods and parameters used in coexpression network construction are detailed in the SI methods. After building each coexpression network, we identified gene modules within each network, which are groups of genes with similar patterns of expression as determined by a topological overlap matrix [43,54].

We initially quantified the overall preservation of the brain's coexpression networks across different social contexts. The preservation of two networks is a measure of how much the topology of the network, and the connections between genes within the network, change across conditions, here mating and social contexts. We measured network preservation based on a differential analysis of eigengene network[15,45], which are coexpression networks between module eigengenes. This analysis relies on estimating the conservation of module eigengenes in each treatment network relative to a consensus network. Eigengenes are calculated as the first principal

component of the gene expression data for each module[46], reflecting the gene expression profile of each module. We included the variance in gene expression explained by each module's eigengene in Table S1. This analysis integrates intramodule, as well as intermodular preservation[45,46], and may thus reflect differential regulation of pathways and biological functions in the neurogenomic response triggered by different social contexts.

We focused on $D_{Preserv}$, an average measure of the preservation between all pairs of modules. Larger values of $D_{Preserv}$ reflect stronger preservation between these pairwise correlations across the two networks, and therefore little network rewiring. We first evaluated the preservation between the coexpression networks in the two male evaluation treatments (Attractive vs Dull mating contexts), and then between each of the male treatments and the social interaction, or female, treatment (Attractive vs Female, and Dull vs Female).

Our analysis revealed overall low preservation values between Attractive and Dull treatment networks in both the optic tectum and the telencephalon. Average $D_{Preserv}^{Att,Dull}$= 0.70, range = 0.66-0.72 in the optic tectum (see further details below; Fig. S1), and average $D_{Preserv}^{Att,Dull}$= 0.74, range = 0.69-0.81 in the telencephalon; Fig. S2). The reported range corresponds to the $D_{Preserv}$ values calculated over multiple combinations of module identification parameters (details below and in methods) to ensure our findings are not biased toward a local parameter space optimum (Table S2).

The parameters used to construct the coexpression networks and identify network modules can affect both size and connectivity scores of identified gene modules, and therefore their preservation across networks. In order to ensure that our results are robust to the combination of parameters used, we tested a range of values for the following parameters: i) method for cluster identification, ii) sensitivity to split clusters ("DeepSplit"), iii) cutting height of dendrogram to identify modules and iv) the merging height when merging modules with similar expression values. We recalculated $D_{Preserv}$ using multiple value combinations for these parameters (26 combinations,

Tables S2, S3 and S4). Variation in $D_{Preserv}^{Att,Dull}$ (Table 1, S2) confirms the low preservation values we find here are a robust to various parameter values. The same is the case for $D_{Preserv}^{Att,Fem}$ *and* $D_{Preserv}^{Dull,Fem}$ despite higher variability in some cases, particularly for $D_{Preserv}^{Dull,Fem}$ in the telencephalon (Table 1, S3 and S4).

A previous cross-species study of neural gene expression rewiring based on the similar methods [45] found far higher preservation values between human and chimpanzee ($D_{Preserv}^{human, chimpazee}$= 0.93). However, this study was based on expression data from roughly 4000 genes, far fewer than in our study. In order to ensure that the lower preservation values we observe are not a consequence of comparing much larger coexpression networks, we repeated the network construction and differential analysis of eigengene network using only the 5000 genes with the highest variance across samples (Table S2). Although the $D_{Preserv}^{Att,Dull}$ increases slightly in some parameter combinations, the effect was on average less than 1% of the value for the whole network. This suggests that the difference in the number of genes used to build these coexpression networks does not account for the far lower network preservation we observe across behavioral states, and that mating context produced a high degree of regulatory network rewiring within the female brain.

We next evaluated network rewiring in response to social contexts beyond mating. We calculated network preservation between each of the male treatments and the general social interaction (female) treatment. Average $D_{Preserv}$ scores were similar to those we observe between mating contexts, with average $D_{Preserv}^{Att,Fem}$ = 0.74 in the optic tectum and 0.70 in the telencephalon (range = 0.69 - 0.82 and 0.69 - 0.80 in the optic tectum and telencephalon respectively, depending on the network construction parameter combination; Table 1, S3). Similarly, average $D_{Preserv}^{Dull,Fem}$ = 0.73 (range 0.68-0.86; Table 1, S4) in the optic tectum and 0.70 (0.55-0.92; Table S4) in the telencephalon. An ANOVA revealed significant differences across the pairwise comparisons (F-ratio=4.85, P-value=0.011), driven by pairwise comparisons involving the attractive treatment

(*Att-Dull* and *Att-Fem*) in both brain tissues (*post hoc* Tukey test P-value=0.01 in both brain components, Fig. S3). Despite having overall low preservation in both tissues, $D_{Preserv}^{Att,Dull}$ had the lowest values in the optic tectum, but the highest in the telencephalon (Table 1; Fig. S3), likely a consequence of the differences in function between these two brain components.

Module rewiring across social contexts

In order to better understand the basis for the high level of network rewiring we observe, we identified those modules within the networks which differ most drastically across networks, thus driving the low preservation we observed in the previous analysis. These modules theoretically represent those biological processes and pathways that are flexibly recruited during exposure to contrasting social stimuli. Here, we used two different measures of module preservation, *Preservation $Z_{summary}$* and *Preservation medianRank*[46]. Unlike differential analysis of eigengene network, which evaluates preservation of the entire network, these measures do not rely on identifying consensus modules, but rather focus on calculating the preservation of each module between two treatment networks[46] (Fig. S4 illustrates differences in the identified modules across contexts and brain regions). Preservation statistics allow us to determine whether genes that are densely connected in the modules of one network remain equally connected in another treatment network.

We first compared Attractive vs Dull, identifying three modules in the optic tectum and five in the telencephalon with *Preservation $Z_{summary}$* < 2 (modules $OT_{AvsD}1$-3, $T_{AvsD}1$-5), a threshold previously established to identify non-preserved modules between networks [46]. Although *Preservation $Z_{summary}$* is sensitive to module size, these modules also had the highest *Preservation medianRank,* a measure far less sensitive to module size (Fig. S5, Table S5), confirming their lack of preservation between the Attractive and Dull coexpression networks. We then repeated this procedure to

identify non-preserved modules between the Attractive vs Female, and the Dull vs Female treatments. In both these pairwise comparisons, we identified 4- 6 non-preserved modules (Tables S6, S7). We determined gene overlap between all non-preserved modules to establish whether the same modules were being identified across all three pairwise comparisons. We found substantial overlap between modules $T_{AvsF}1$ and $T_{AvsF}2$ and modules $T_{AvsD}1$ and $T_{AvsD}4$ in the telencephalon (one tail Fisher test P-values <0.01; Table S8), suggesting we are indeed dealing with the same or very similar modules in the different analysis. These two modules represent gene biological pathways that could regulate processing of social stimuli in different contexts.

In order to investigate what these biological functions are, we identified the Gene Ontology (GO) terms and KEGG pathways enriched within these non-preserved modules (Fig. S6, Table S9; Supp. Dataset 1, 2). Of particular note are GO terms associated with cognition as well as multiple terms highly relevant to neuronal processes of social behavior (i.e. regulation of NMDA & AMPA glutamate receptors, GABAergic synaptic transmission, Wnt signaling, JNK cascade). Moreover, we found enrichment for several KEGG pathways relevant to behavior. Amongst these, the GnRH and GnRHR signaling pathways, which have been linked to preferences for familiar males in medaka (*Oryzias latipes*) and social learning in swordtail fish (*Xiphophorous hellerii*) [55,56], and hormone signaling pathways that mediate the neuroendocrine changes related to mating decisions (i.e. Oxytocin and Estrogen signaling pathways, Progesterone-mediated oocyte maturation).

Identification of differentially coexpressed gene pairs

After evaluating overall network preservation and identifying non-preserved modules within the networks, we used a complementary analysis to determine which specific gene pairs within modules are differentially co-expressed (DC), driving the network rewiring we observe. These are pairs of genes that significantly change in correlation across social contexts (Fig. 1B), and may

reflect differences in gene expression, mRNA stability or splicing[41] and point to the biological pathways that change across social contexts. We used differential coexpression analysis in BFDCA[47], a complementary approach to the more commonly used differential expression analysis, which identifies loci with different regulatory connections within the network independent of whether they are differentially expressed[17,47,57]. BFDCA uses gene expression levels to identify genes that have significantly different correlation values between two treatments, referred to as differentially coexpressed (DC) gene pairs (Fig. 1B). For more details see SI methods and Wang et al (2017).

Comparing the two male treatments, we identified 567 DC gene pairs in the optic tectum and 30 in the telencephalon (Supp. Dataset 3). These DC gene pairs passed permutation-based significance thresholds (see details in SI methods), and were thus classed as displaying significantly different correlations. Some of these genes have multiple significant DC connections with other genes, suggesting they may play an important role in modulating male evaluation (Table S10, Fig. S7). Our data indicates there is a more extensive rewiring of the coexpression network at the sensory-processing level, in the optic tectum, than at the decision-making level (telencephalon) when a female is evaluating the two different types of males (Fig. S7). This suggests that sensory processing of different social stimuli tested here involves a larger number of genes and thus portions of the network, while the early decision-making process generated by those stimuli is initiated by fewer genes of large effect.

GO term and KEGG pathway enrichment tests confirm the functional relevance of the DC gene pairs we identified, revealing an overrepresentation of genes associated with multiple metabolic processes, as well as visual transduction and cognition among optic tectum DC gene pairs (Fig. S8). In the telencephalon, DC gene pairs were significantly enriched for terms associated with learning and memory, cognition, visual behavior and multiple terms related to synaptic transmission (Fig. S8, Supp. Dataset 3). Moreover, terms associated with pregnancy and control of hormone levels

suggest the differential regulation of pathways associated with the neuroendocrine and receptivity changes that occur in females with the decision to mate (Fig. S8). The DC gene pairs we identified are also part of genetic pathways highly relevant to behavior, including KEGG pathways associated with many neuropsychiatric disorders such as Alzheimer's disease (summarized in Table S11).

Next, we identified DC gene pairs in the two mating treatments relative to the social control condition in which females were exposed to another female. Surprisingly, we find no DC gene pairs common to all three pairwise treatment comparisons (Fig. 2). This suggests that there are no obligatory regulatory connections that need to be involved in all social contexts. It is worth highlighting that this result contrasts with our previous findings of a group of differentially expressed (DE) genes that are common to all three comparisons, suggesting they contribute to behavioral control by changing their expression level in a context-dependent manner[11]. However, we did identify genes in each brain region that belong to all non-preserved modules identified in all three pairwise treatment comparisons from our previous analysis (Table S8D and S8H). Finding no overlap with BFDCA might reflect limitations of the method, or it could be a consequence of the stringent permutation procedure we used to determine BFDCA gene pair significance. Taken together, our analysis suggests there are genes that change their expression and their network connections across all social contexts, while others are only recruited in response to specific social stimuli.

Finally, we investigated the genes that could be regulating rewired modules. We used the human TransFac database[58] of transcription factor motifs (TF motifs) to predict the transcription factor motifs that are significantly enriched in non-preserved modules, with the caveat that humans and guppies are distantly related species. We found each of the non-preserved modules is predicted to be regulated by a different combination of transcription factors, including multiple transcription factors relevant to behavior, cognition, memory and learning (see complete list in Supp. Dataset

4). Importantly, many of the predicted TF motifs correspond to genes identified as DC gene pairs with BFDCA (Table S12), confirming their role as regulators of the differences in the neurogenomic response triggered by the different social stimuli we tested. Moreover, many of the DC gene pairs are also part of non-preserved modules suggesting these rewired connections are the basis of differences in expression within these modules. This is particularly striking in the optic tectum, where 132 genes that form DC gene pairs are found within non-preserved module OT1 (Table S12). Two genes, *KLF5* and *TCF12*, are particularly noteworthy. These two genes are differentially coexpressed in the optic tectum, and were also identified in our previous study [11] as differentially expressed transcription factors, with a role modulating male evaluation in guppy females with and without female preferences. More details into the overlap between genes found to be differentially expressed in our previous study [11] and DC gene pairs can be found in Table S13.

Subnetworks activated in a context-dependent manner

In order to illustrate how these DC gene pairs could represent subnetworks that are differentially activated in response to different mating and social stimuli, we analyzed the subnetworks of genes *GRIN1* and *GLUL* in more detail. These genes were identified among the genes with the highest number of DC connections in the optic tectum and telencephalon respectively (Table 2), and change their connections to other genes in a context-dependent manner (Fig. 3). *GRIN1* and *GLUL* have known roles regulating synaptic processes involved in learning and memory, and have previously been associated with social behavior and neuropsychiatric disorders[59-62]. *GRIN1* in particular, encodes the glutamate ionotropic receptor NMDA type subunit 1, which has previously been associate with mate preference in poecilids after pharmacological manipulation[62].

GRIN1 is differentially coexpressed with two genes, *TUBB4b* and *CLCN6* (Fig. 3A), and is among the genes that belong to all the non-preserved modules identified across comparisons in the optic

tectum (Table S8D), providing additional evidence of its role in the processing of social stimuli. The *GRIN1* subnetwork differs whether a female is evaluating an Attractive vs a Dull male. Remarkably, the connections between genes in the *GRIN1* subnetwork are completely absent in a non-mating context, when a female is exposed to another female (Fig. 3B).

We see a similar example of extensive rewiring with the *GLUL* subnetwork in the telencephalon. This gene has significant differential correlations with four other genes (Fig. 3C) that potentially trigger drastic rewiring (Fig. 3D). We see a very high number of connections in the telencephalon within this subnetwork when a female evaluates an attractive male that she would potentially chose to mate with. We can see the extent to which these connections change between mating contexts in Fig. 3D. Importantly, many gene connections are only present in mating contexts, and the *GLUL* subnetwork has very few connections in our social (Female) treatment, making this another example of gene connections that are only associated with mate evaluation (Fig. 3D).

DISCUSSION

Using systems biology methods novel to the study of behavior, we found remarkably low network preservation across social contexts which can be interpreted as an extraordinary degree of flexibility in the transcriptional state of the brain across various mating and social stimuli. Our preservation measures among different social contexts are far lower than similar analyses done between different species, which obtained values of brain network preservation between human and chimpanzee of $D_{Preserv}^{human, chimpazee} = 0.93$ in the absence of any social interactions (data from [41] also analyzed in [45]). Our results suggest that the topology and gene connections of the optic tectum and the telencephalon coexpression networks change more dramatically in female guppies evaluating different types of males, than between humans and chimpanzees in the absence of social stimuli.

It is important to consider a few caveats when interpreting the low network preservation values we find relative to this previous human-chimpanzee study[41]. The first issue to note is that both studies are based on different gene expression quantification techniques. Here we use RNAseq, while Oldham et. al[41] used microarrays, with predetermined transcripts, that could increase $D_{preserv}$ estimates compared to RNAseq. However, given the high correlation between these technologies in gene expression quantification across the majority of the expression spectrum[63,64], it is unlikely that this accounts for the majority of the difference in $D_{preserv}$ values we observe. Additionally, running the analysis under diverse conditions confirmed these results are robust to changes in the parameter combination used in the analysis. A similar differential eigengene network analysis revealed overall preservation values between the eigengene networks of multiple mouse tissues (liver, brain, muscle and adipose tissue) ranging between 0.85 and 0.93, and 0.94 between male and female livers[45]. Our surprising findings indicate greater intraspecific plasticity in neurogenomic response than the observed baseline differences between species. Moreover, these findings provide an estimate of how large the scale of gene coexpression network remodeling can be across the different neurogenomic states triggered by different social stimuli, and thus the complexity of the brain's response to these various stimuli.

Further analysis suggests the preservation between the coexpression networks of each mating/social context we tested is driven by a handful of modules. Two of these modules are identified as having low preservation in both pairwise comparisons involving the Attractive male treatment. This finding suggests these modules group the biological processes that differentiate the processing of an attractive male stimulus from the other stimuli we tested. Gene Ontology (GO) and KEGG pathway analysis indicate that these modules are enriched in behaviorally relevant terms associated with learning and memory, mating behavior, cognition and multiple biological pathways highly relevant to neuronal processes relevant to social behavior (Fig. S6, Table S9 and Supp. Dataset 1 GO and 2 KEGG). These findings are mirrored by recent reports of a similar over-

representation of learning and memory genes in a neurogenomic study of poecilid mate preference[65]. Our results are consistent with previous studies suggesting neurogenomic states at the basis of different behaviors involve specific modules within the brain coexpression network, rather than genes interspersed throughout the network [21,66,67]. Although most of these studies rely on different methodologies, our findings contribute further evidence to this model and our understanding of the way social behavior is controlled in the brain.

Although rare, the results of existing studies using differential coexpression in the study of behavior [20,29] are consistent with our findings in that specific network modules group genes with the most significant changes in connectivity. These studies find concordant changes in connectivity associated with the studied behaviors across various genetic lines, despite having found limited and/or inconsistent changes in the gene expression [11]. Like ours, these results speak to the value of differential coexpression analysis in behavior, as a complementary approach to the more frequently employed differential expression methods. One of these studies [20,29], which focused on catalepsy behavior in mice, is promising as it links specific polymorphisms to the gene connectivity changes triggered by a specific behavior in the brain coexpression network. Aside from identifying potential regulators of the studied behavior, this work once more validates the biological significance of these differential coexpression methods and their potential in the study of behavior. Here, a word of caution is appropriate since our sample size is on the lower range and could be limiting our power to detect all connectivity changes within these coexpression networks.

Identifying genes that rewire their connections across mating conditions brings to light loci that are important in mediating mating behavior. These DC genes could potentially be the regulators of the pathways that are differentially activated when the brain produces different behaviors. Here, we found subnetworks that are only recruited in a mating context, not during a general social interaction, as we illustrated with the *GRIN1* and *GLUL* examples. These are examples of gene pathways that are only recruited in a context dependent manner during different social

interactions. Moreover, identifying DC genes that are only connected to other genes in mating or non-mating contexts provides important insight into the mechanisms by which the brain mediates the response to different social stimuli, such as mating vs non-mating social encounters. Our study suggests an important role of gene connectivity changes in the brain's neurogenomic response to social stimuli, and illustrates the power and potential of differential coexpression analysis in the study of behavior and evolutionary biology. These methods go beyond differential expression to show how the connections between genes change in different conditions and offers a view of important gene players that cannot be detected when studying differential expression[11].

LIST OF SUPPLEMENTARY MATERIALS

SI Methods

Figs. S1 to S8

Tables S1 to S13

Captions for datasets S1 to S4

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FIGURE LEGENDS

Figure 1: Methodology overview.

Using gene expression (RNAseq) data for each treatment, we investigated network rewiring between treatments by building coexpression networks for each treatment. We then performed two types of analysis. (A) We initially evaluated whole network and module preservation. Then, (B) we identified differentially co-expressed (DC) gene pairs using the BFDCA algorithm. (A) Example of co-expression network produced by calculating correlations between genes based on their expression. Each node corresponds to a gene and its size is proportional to the number of genes it is connected to. (B) We used the BFDCA algorithm [47] to identify DC gene pairs. Using the gene expression levels for all genes that vary between the two treatments, BFDCA calculates Bayes factors and identifies DC gene pairs. Significant DC pairs are selected by filtering out DC pairwise correlations between genes with Bayes factor values below a threshold, then calculating each DC pair's weight to reflect its importance. We used a permutation approach to select significant DC gene pairs. Significant DC gene pairs belong to several categories according to how the correlations of both genes differ between treatments. The original categories from Wang et al. (2017) were 're-wiring', "cross" and "shift"; here we replaced "re-wiring" by "correlation loss" to avoid confusion with the more general use of rewiring we adopted thought the manuscript. For insets illustrating these categories, X and Y-axis represent expression levels for differentially coexpressed genes A and B respectively.

Figure 2: Preservation statistics of Attractive modules in Dull treatment samples for (A) the optic tectum and (B) the telencephalon.

Venn diagram showing the number of significant DC gene pairs determined to be significant after conducting permutations in each pairwise treatment comparison. In each case the top number in black corresponds to DC gene pairs in the optic tectum, and the lower number in blue corresponds to the number of telencephalon DC gene pairs. Arrows and text are used to facilitate the biological interpretations of each area of the Venn diagram.

Figure 3: Examples of differential correlations (DC) for genes of interest (A-C) and corresponding network re-wiring (B-D) across social contexts in optic tectum and telencephalon networks.

In the optic tectum we have highlighted differential correlations of gene *GRIN1,* found to be differentially correlated across treatments (A-B) and gene *GLUL* in the telencephalon (C-D). Panels

A and C represent correlations between genes of interested and other genes with which we found significant differential correlations between treatments. The axes represent expression levels for each gene, each point corresponds to a sample and the ellipses represent the 95% contours of the bivariate normal density estimated for each treatment color-coded according to legend. Asterisks of the appropriate color next to ellipses mark correlations higher than 0.65.

B & D: To construct the subnetworks of each gene, its neighbors were selected on the global coexpression network build with all samples from all treatments. This way it is possible to identify all the neighbors for *GRIN1* and *GLUL* that have significant correlation with these genes in any of the behavioral treatments. These sets of genes were then used to build subnetworks separately for each treatment (B-D). Only correlations higher than 0.65 are shown, with positive correlations represented by green edges and negative correlations by red edges. Genes highlighted in blue were found to have significant DC with the genes of interest in each tissue, and thus correspond to the genes in scatterplots A and C. Size of the gene nodes is proportional to the gene connections it has within the network (degree).
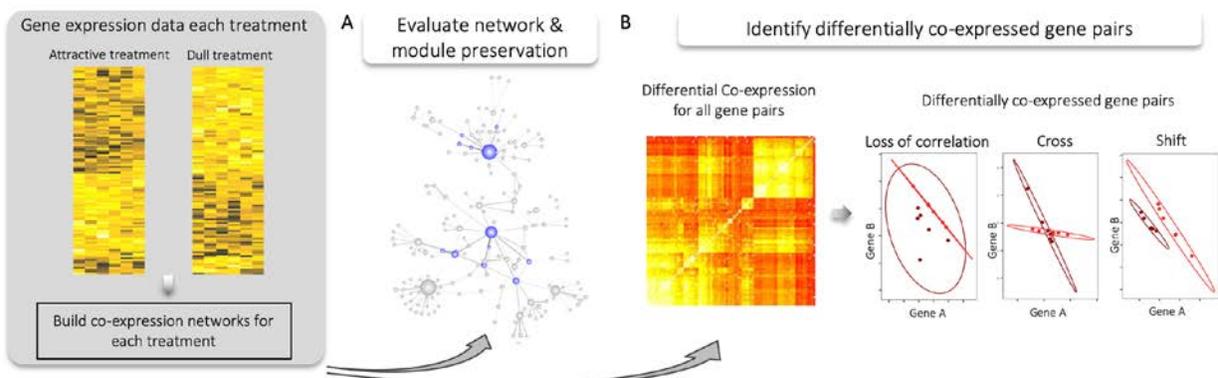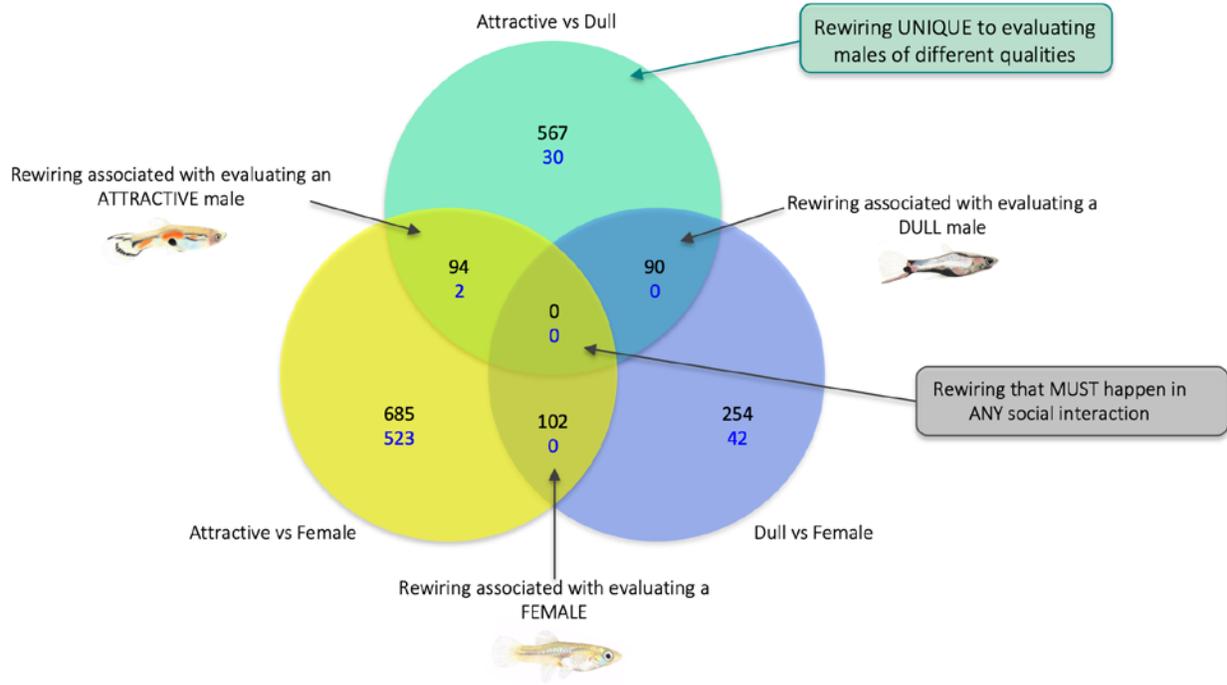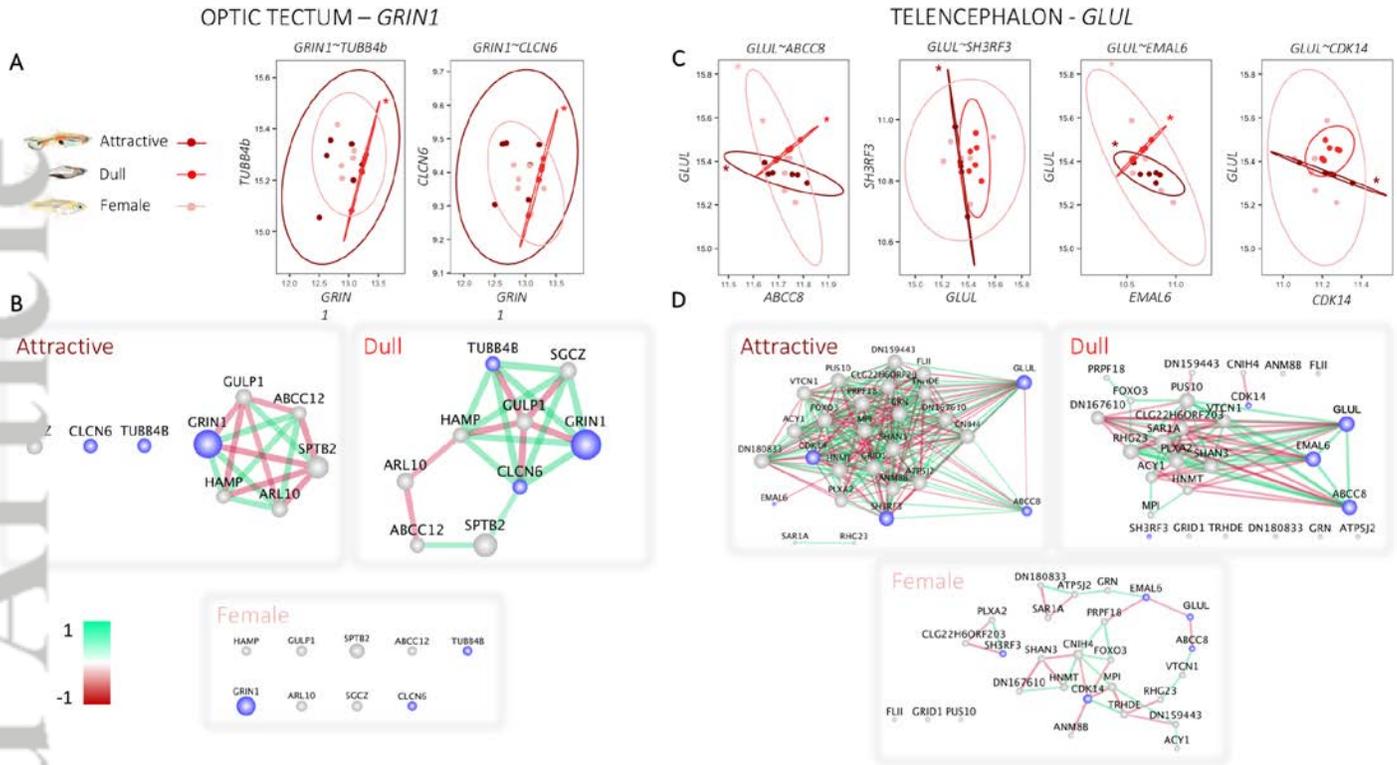
FIGURES

FIGURE 1

FIGURE 2

FIGURE 3

OPTIC TECTUM – *GRIN1*

TELENCEPHALON - *GLUL*

TABLES

Table 1:

Table of *Dpreserv* values

	OPTIC TECTUM				TELENCEPHALON			
	$D_{Preserv}$ estimates		Pairwise comparison ANOVA$^\delta$		$D_{Preserv}$ estimates		Pairwise comparison ANOVA$^\delta$	
	Average $D_{Preserv}$	Variability*	F-ratio	P- value	Average $D_{Preserv}$	Variability *	F-ratio	P- value
Attractive-Dull	0.70	0.66-0.72			0.74	0.69-0.81		
Attractive-Female	0.74	0.64-0.82	4.85	0.01*	0.70	0.64-0.80	5.16	0.008**
Dull-Female	0.73	0.68-0.86			0.70	0.55-0.92		

* Variability when running the model over a wide range of module detection parameter combinations.

$^\delta$ ANOVA performed with the purpose of determining whether there are significant differences in our estimates of $D_{Preserv}$ across the three pairwise comparisons, Attractive vs Dull, Attractive vs Female and Dull vs Female in both brain tissues (further details in Fig. S3).