Neurogenomics of Behavioral Plasticity

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Abstract

Across animals, there is remarkable diversity in behavior. Modern genomic approaches have made it possible to identify the molecular underpinnings of varied behavioral phenotypes. By examining species with plastic phenotypes we have begun to understand the dynamic and flexible nature of neural transcriptomes and identified gene modules associated with variation in social and reproductive behaviors in diverse species. Importantly, it is becoming increasingly clear that some candidate genes and *gene networks* are involved in complex social behaviors across even divergent species, yet few comparative transcriptomics studies have been conducted that examine a specific behavior across species. We discuss the implications of a range of important and insightful studies that have increased our understanding of the neurogenomics of behavioral plasticity. Despite its successes, behavioral genomics has been criticized for its lack of hypotheses and causative insights. We propose here a novel avenue to overcome some of these short-comings by complementing "forward genomics" studies (i.e., from phenotype to behaviorally relevant gene modules) with a "reverse genomics" approach (i.e., manipulating novel gene modules to examine effects on behavior, hormones, and the genome itself) to examine the functional causes and consequences of differential gene expression patterns. We discuss how several established approaches (such as pharmacological manipulations of a novel candidate pathway, fine scale mapping of novel candidate gene expression in the brain, or identifying direct targets of a novel transcription factor of interest)

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C.R. Landry and N. Aubin-Horth (eds.), *Ecological Genomics: Ecology and the Evolution of Genes and Genomes*, Advances in Experimental Medicine and Biology 781,
DOI 10.1007/978-94-007-7347-9_8,

can be used in combination with the analysis of the accompanying neurogenomic responses to reveal unexpected biological processes. The integration of forward and reverse genomics will move the field beyond statistical associations and yield great insights into the neural and molecular control of social behavior and its evolution.

Keywords
Transcriptomics • Reverse genomics • Neuroethology • Social behavior • Dispersal • Mate choice • Evolution

8.1 Introduction

Across the animal kingdom, there is remarkable diversity in naturally occurring behavioral phenotypes. Many animals live in complex social environments, and they make decisions based on the context of their interactions with other individuals. How do they make these decisions, and why do they behave the way they do are questions that have long fascinated biologists (Tinbergen 1963). A recent review by O'Connell and Hofmann (2011a) outlines a variety of ways in which these questions can be addressed by combining genomic and evolutionary approaches with studies examining brain and behavior. Modern genomic techniques such as *microarrays* (see the Glossary for definitions of italicized terms) and, more recently, *next-generation sequencing* have made it possible to examine the molecular underpinnings of plasticity in animal behavior and decision-making as well as their evolution (Hitzemann et al. 2013). By examining neural *transcriptomes* of polymorphic species we have begun to understand the dynamic and flexible nature of genome activity in the brain and identified *gene modules* (set of co-regulated genes or proteins (Segal et al. 2004)) that are associated with variation in social and reproductive behaviors in diverse species (O'Connell and Hofmann 2011b). While it is increasingly clear that some candidate genes and gene networks are involved in complex social behaviors across even divergent species (O'Connell and Hofmann 2011b; Toth and Robinson 2007), few comparative transcriptomics studies have been conducted to test this notion of conserved molecular pathways on a genomic scale.

Behavioral genomics has clearly transformed our understanding of social plasticity, yet the field has also been criticized for its apparent lack of concrete hypotheses and the uninformative gene lists that often result from these studies. While it is indeed relatively easy to obtain a wealth of transcriptional information, identifying the genes or gene networks that are causal in the behavioral context under study is much more challenging. In the same manner that geneticists advance the field by using reverse genetics (Alonso and Ecker 2006), it is thus becoming increasingly important that these "forward genomic" studies are followed up with "reverse genomic" studies to examine the functional causes and consequences of differential gene expression patterns. In other words, once novel candidate genes or pathways have been identified, we must use experimental tests on a genomic scale to further dissect the contribution of each gene to the behavioral phenotype.

Here, we discuss forward and reverse genomic studies that have shed light on various aspects of social behavior and its underpinnings and suggest promising avenues for future research into the evolution of neuroethological systems. There are many examples of forward genomic experiments and a dearth of reverse genomic experiments, which we argue are necessary for examining causality and function. We highlight several studies that have applied a "reverse genomics" approach successfully in diverse model systems, complementing approaches such as pharmacological manipulations of a novel candidate pathway, distribution mapping of novel candidate gene expression in the brain, or identification of direct targets of a novel transcription factor of interest with transcriptomics (Fig. 8.1). The

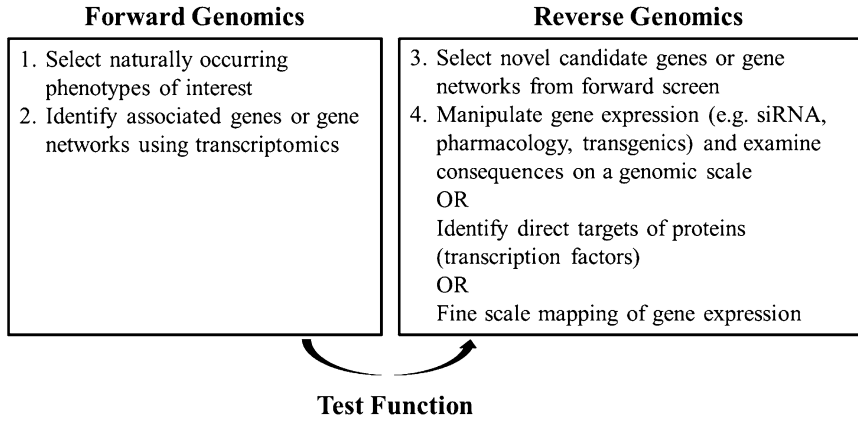

Fig. 8.1 Forward and reverse genomics of behavioral plasticity. *1*. Forward genomic approaches begin with the selection of two or more phenotypes for comparison. *2*. Then gene expression differences are compared on a genomic scale to identify genes and gene networks that are associated with the naturally occurring behavioral phenotype. *3*. Reverse genomics begins with the selection of novel candidate genes, gene networks, or pathways based on the gene expression analysis. *4*. To better understand the function of the observed gene expression patterns, one can manipulate gene expression, identify DNA/protein interactions, or examine brain region specific differences

need to examine the neurogenomic responses that result from these perturbations is increasingly becoming clear. The combination of forward with reverse genomics will move the field beyond statistical associations and yield great insights into the neural and molecular control of social behavior and its evolution.

8.2 Functional Genomics of Neural and Behavioral Plasticity

Plasticity in the nervous system comprises the functional and structural changes in information processing after the initial formation of neuronal contacts. When approached from an integrative perspective, the analysis of these mechanisms usually begins by describing and analyzing the neural, endocrine, and behavioral traits that can potentially be realized by an organism or in a population depending on environmental conditions. We can distinguish several (often overlapping) time scales on which plasticity can occur in response to social or environmental stimuli (Hofmann 2003): Changes that occur in real time (e.g., modulation, learning/memory formation) via variation in neural and/or hormonal activity; slower changes that involve regulation of gene expression as well as possible structural and physiologic changes; and changes that can alter developmental trajectories and shift neural functioning throughout life history, even in adult animals (e.g., seasonal and use-dependent changes). A number of studies have integrated concepts from neurobiology, ethology, and evolutionary biology with powerful genomic technologies in order to gain a more comprehensive understanding of the roles that genetic and environmental factors play in neural and behavioral plasticity.

8.2.1 Alternative Reproductive Tactics

Organisms that share the same genotype can develop into divergent phenotypes, depending on environmental conditions (Brockmann 2001; Ross 1990). Atlantic salmon (*Salmo salar*) exhibits extreme alternative life histories and *reproductive tactics* based on their growth rate and duration as juveniles. Young males of the same age can be found either as mature sneakers or immature males that will be anadromous the next year. Aubin-Horth and colleagues (2005) hypothesized that brain gene expression patterns would vary considerably between age-matched mature males (sneakers), immature males (future

anadromous males) and immature females. Specifically, these differences would correspond to organism-level phenotypic variation between divergent life history and developmental trajectories. A microarray analysis of whole brain transcriptomes revealed that 15 % of ~3,000 genes examined were differentially expressed in the brains of the two male types, many of which are involved in processes such as growth, reproduction, and neural plasticity. Interestingly, consistent patterns of gene expression were found for individuals of the same reproductive tactic despite the potentially high individual variation that is often associated with genomic studies on wild caught animals. Notably, gene expression patterns in immature males were quite different both from immature females and mature sneakers; this pattern indicates that delayed maturation and sea migration, the 'default' life cycle, may actually result from an active inhibition of development into a sneaker (Aubin-Horth et al. 2005, 2009).

Like the Atlantic salmon, the ocellated wrasse, *Symphodus ocellatus*, is another fish species in which males display plasticity in life history trajectory and reproductive tactic. *S. ocellatus* males engage in one of three alternative tactics during a reproductive season: nesting, satellite, and sneaker. While males utilize a single tactic per reproductive season, reproductive tactic is plastic because males can transition to other tactics between seasons and thus have multiple potential life history trajectories depending on early growth prior to their first winter or first reproductive year. Satellites and sneakers spawn parasitically in nesting males' nests, but only nesting males provide parental care. Nesting and satellite males show transient cooperative defense of nests against sneakers. To better understand the neuroendocrine and genomic mechanisms that give rise to these dramatic differences in phenotype, Stiver and colleagues (in prep) analyzed neural gene expression profiles and circulating sex steroid hormone levels in these three male phenotypes and in females. Multivariate analyses of the genes that were differentially expressed between any two phenotypes revealed striking similarities and differences in expression profiles between phenotypes. Specifically, brain transcriptomes of satellites and females were most similar to each other, while nesting and sneaker males were most dissimilar from each other and from the other phenotypes. Sneakers showed more total expression differences, whereas nesting males showed higher magnitude expression differences. Based on work by Aubin-Horth et al. (2007), Aubin-Horth et al. (2005), Renn et al. (2008), Schumer et al. (2011), AVT and parvalbuminm RNA levels were expected be highest in the dominant, nesting males, but AVT was highest in the female, and parvalbumin was highest in the satellite males. Ribosomal-, histone-, and proteasome-related genes, which were expected to correlate with future growth (Alonzo et al. 2000; Renn et al. 2008) were indeed up-regulated in sneakers and satellite males.

With respect to circulating sex steroid hormones, 11-ketotestosterone (but not testosterone) was highest in nesting males, while estradiol was highest in females. Overall, these genomic and endocrine findings reveal the surprising extent to which neural gene expression patterns vary across reproductive tactics, providing important insights into the molecular mechanisms underlying variation in cooperative and reproductive behavior (Stiver et al. in prep).

8.2.2 From Nurse to Forager

Some animals undergo fascinating changes in brain and behavior across their lifetime. The non-reproductive workers of honeybee (*Apis mellifera*) societies provide a compelling example as they transition through a series of distinct behavioral tasks as they age (polyethism). Worker bees begin their adult lives tending to within-hive chores such as nursery and queen care and then transition to the role of a forager. This age-related transition to foraging is associated with dramatic changes in brain morphology and brain gene expression. For example, the mushroom bodies, a region in the insect brain associated with complex social behavior and memory (Haehnel and Menzel 2012), increase in size (Fahrbach 2006). There are also substantial changes in gene expression (>85 % of approximately 5,500 genes showed

differences) associated with the transition from nurse to forager that are largely independent of age-related changes. Principal component analysis revealed discrete influences of age, behavior, genotype, environment, and experience (Whitfield et al. 2006). Interestingly, the hive bee to forager transition is accompanied by changes in genes related to energy metabolism and genes driven by the actions of juvenile hormone, highlighting the importance of hormones in driving neural plasticity (Ament et al. 2010). Inspired by findings in *Drosophila* (Osborne et al. 1997), Ben-Shahar et al. (2002) showed that the age-related transition from hive worker to forager is associated with increased expression levels of the foraging gene (for). Furthermore, treatment with a guanosine 3′, 5′-monophosphate (cGMP) -dependent protein kinase (PKG) that is encoded by *for* caused foraging behavior (Ben-Shahar et al. 2002).

8.2.3 Social Hierarchies

It is well known that behavior and physiology are regulated by both environment and social context, and an important study by Renn and colleagues demonstrated that neural gene expression is regulated by social environment (Renn et al. 2008). The authors used the African cichlid fish *Astatotilapia burtoni,* a model system for the study of how social interactions regulate neural and behavioral plasticity (Hofmann 2003; Robinson et al. 2008). *A. burtoni* males are either socially dominant, territorial, reproductively active, and brightly colored or subordinate, non-territorial, reproductively suppressed, and cryptically colored. Amazingly, these phenotypic differences are reversible, and males ascend and descend many times during their life. Renn et al. examined whole brain gene expression in dominant and subordinate males as well as in brooding females, and integrated the genomic data with quantitative behavioral measures. Using this integrative approach, the authors identified co-regulated gene sets (gene modules) that are significantly associated with either *dominance* or reproductive state. While the regulation of neuroendocrine genes was predicted from previous research, the results also revealed unexpected and novel roles for two classic neurotransmitter systems (GABA and glutamate/kainate) in mediating behavioral plasticity. Also, the application of the Gene Ontology framework (Ashburner et al. 2000) underscored the importance of hormonal regulation and highlighted the hitherto under-appreciated roles of cytoskeletal components and neuronal remodeling activity in addition to neurochemical pathways. Importantly, the authors found a high degree of individual variation in expression levels of genes that are differentially regulated between these phenotypes even though the dominant and subordinate phenotypes are robustly defined. These results demonstrated the molecular complexity in the brain associated with different social phenotypes, including gene modules that underlie reproduction and submissive behavior (Renn et al. 2008). Taken together, this genome-scale analysis of molecular systems in the brain identified complex patterns of gene expression that are associated with a socially regulated switch in behavioral phenotype.

As a follow up study, Huffman and colleagues (2013) designed an experiment to analyze the role of aromatase, the enzyme that converts testosterone into estradiol, in mediating aggression and reproductive behavior in male *A. burtoni*. Using quantitative radioactive *in situ* hybridization, the authors found that subordinate males have higher aromatase expression than dominant males in the magnocellular and gigantocellular regions of the preoptic area. Then, they pharmacologically inhibited aromatase activity by giving intraperitoneal injections of fadrozole (FAD) to dominant males and found that FAD treatment decreases aggressive, but not reproductive, behaviors compared to saline controls. Furthermore, they found that circulating estradiol levels decreased while testosterone levels increased in response to FAD treatment. Moreover, FAD-treated males had increased aromatase expression in the gigantocellular portion of the preoptic area (POA), possibly a compensatory response. Together, these results suggest that aromatase promotes aggression in *A. burtoni* males through actions in the preoptic area (Huffman et al. 2013). While this study

did not examine the genomic response to FAD treatment, it did test for function associated with the significant correlations found between dominance behavior and aromatase gene expression identified by Renn et al. 2008.

In an elegant study on the molecular basis of social dominance, Aubin-Horth et al. (2007) used the cooperatively breeding African cichlid *Neolamprologus pulcher* to identify brain gene expression profiles associated with aggression and dominance behavior independent of sex. In this species, dominant individuals (males and females) display similar behaviors, have high testosterone levels and have high brain arginine vasotocin expression when compared to subordinate helpers, but dominant females have lower levels of 11-ketotestosterone than males. Furthermore, brain gene expression profiles of dominant females are most similar to those of the males (independent of social rank), indicating that dominant breeder females are masculinized at the molecular and hormonal level while being at the same time reproductively competent. By investigating different levels of biological organization, from behavior to hormones and gene expression, this study provided new insights into the mechanisms underlying vertebrate social dominance, and the molecular and endocrine masculinization of the female brain depending on social status is likely not limited to fishes. This finding underscores the need for a comparative approach in a wide range of vertebrates with diverse patterns of social organization to determine where similar molecular and endocrine substrates regulate social life and where they have evolved independently (Aubin-Horth et al. 2007).

8.2.4 Social Defeat

To characterize the neural circuitry and cellular process by which social experience alters the activity of the mesolimbic dopamine pathway, Nestler and colleagues (Berton et al. 2006) used a chronic social defeat paradigm. In this paradigm, a mouse that is repeatedly exposed to a more aggressive individual will display increased anxiety and decreased exploratory behaviors. These depression-like phenotypes are associated with differences in BDNF (brain-derived neurotrophic factor) concentrations in the *nucleus accumbens* (NAcc), a brain region central to processing the salience and rewarding properties of a stimulus. This research has provided good evidence for socially induced remodeling of the physiological, molecular, and cellular mechanism within this mesolimbic dopamine pathway that affects stimulus processing. These modifications included changes in activity of transcription factors, histone modification and DNA methylation, giving rise to short and longer term changes in gene expression (Nestler 2012a). A microarray study from the same group (Krishnan et al. 2007) revealed that resilient mice (i.e., individuals who maintain normal physiological function despite defeat experience) showed selective upregulation of multiple voltage-gated K^+ channel subunits in the ventral tegmental area (VTA; the source of dopamine affecting the NAcc) after chronic social defeat, but maintained low BDNF release from the VTA as in controls. This inspired them to examine the electrochemical properties of the VTA neurons. The increase K+ channel correlated with decreased firing of VTA neurons. Studies like this show the power of integrating electrophysiology with functional genomics and protein assays to better understand behavioral, cellular, and molecular responses to social challenges.

8.3 Molecular Mechanisms of Decision-Making

Animals are confronted daily with social challenges and opportunities where they must make adaptive decisions to ultimately increase their fitness. The brain integrates external social or environmental information with internal physiology by changes in neural gene expression and organization. Variation in neural gene expression patterns can have profound influences on how an individual responds to a stimulus and explains why we see so much diversity in animal behavior between individuals of the same species, across an individual's lifetime, and over

generations. Such molecular changes allow animals to integrate social information into an appropriate behavioral response, orchestrate neural changes that promote reproduction, and respond to social and other cues in ways that ultimately may serve to maximize fitness. In this section we review several studies that examine the rapid changes in neural activity and gene expression that are associated with behavioral decision-making.

8.3.1 Neuroeconomics

We begin this section with a discussion of neuroeconomics, an interdisciplinary field that combines cognitive neuroscience tools and economic theory to study the processes that govern behavioral decision making in the human brain (Fehr and Camerer 2007). Experimental games are often used in this research to measure how the salience of a reward (often monetary) influences a player's behavior. There are many types of games that can be used to study decision-making processes. These games create paradigms on how social status, age, and sex influence social decision making. The prisoner's dilemma is an excellent game theory example that demonstrates why two individuals might cooperate even when it is not in the individual's best interest. Thus, decision making is complex because individuals are motivated not only by personal gains but also by some reward derived from cooperating in certain social situations (Brede 2013). Humans frequently sacrifice material and personal gains to endorse or to oppose societal causes. The neural basis of charitable donation behavior has been the subject of experimental neurogenomic economics studies using a modified prisoner's dilemma paradigm and functional Magnetic Resonance Imaging (fMRI). The players were subjected to fMRI while choosing to donate or not to donate to real charitable organizations. Surprisingly, the mesolimbic reward system was engaged when the player donated to a charity and when the player received a monetary reward, suggesting that that the act of being charitable is itself rewarding. While social neuroeconomic studies have provided the evidence for neural circuits involved in decision making (Moll et al. 2006), they provide little insight into the genetic and genomic underpinnings, and we therefore return to animal model systems.

8.3.2 To Sing or Not to Sing?

A classical method of measuring neuronal responses is through electrophysiological recordings. Such studies often focus on presenting an animal with a behaviorally relevant sensory stimulus and measuring neuronal activity in various brain regions. Songbirds provide a powerful model system in this regard, where songs produced by males vary based on the social context. In the zebra finch (*Taeniopygia guttata*), neuronal activity is markedly different in brain regions involved in song learning when the male sings a song directed at a conspecific compared to undirected song (Hessler and Doupe 1999). However, recording neural activity simultaneously in several nodes of the birdsong circuit of an awake and behaving animal in a naturalistic environment is not feasible in most cases. To determine what brain regions or neuronal populations may respond to a particular social stimulus or which brain areas are active during singing, many researchers therefore use detection of *immediate early genes* (IEGs) as markers of neuronal activity (Jarvis and Nottebohm 1997; Mello et al. 1992). IEGs (e.g., *c-fos*, *jun*, *egr-1*, *arc*; Loebrich and Nedivi 2009) are typically transcription factors that are thought to quickly respond to internal and external stimuli and thus coordinate neuronal plasticity. Dong et al. (2009) expanded this experimental framework using a microarray approach. They showed that exposure to novel song induces rapid expression changes in thousands of genes, many of which are involved in transcription and RNA processing as well as cellular homeostasis. These authors concluded that natural stimuli such as birdsong can result in major changes in the metabolic state of the brain (Dong et al. 2009).

Gene expression studies have also revealed that the transcription factor *FoxP2* is critical for singing in songbirds. Within the song-specialized striato-pallidal Area X, *FoxP2* levels decrease after 2 h. of undirected singing (Teramitsu et al. 2010; Teramitsu and White 2006), and the magnitude of down-regulation is correlated to how much the birds sang (Teramitsu et al. 2010). Hilliard et al. 2012 used this finding as a starting point for examining the genomic differences between singing and non-singing males. The songs of singing males were undirected, presumably to remove any confound caused by the presence of a social stimulus. RNA was extracted from Area X for microarray analysis (Hilliard et al. 2012). In order to look for broad patterns in the dataset, the authors employed weighted gene coexpression network analysis (WGCNA; Zhang and Horvath 2005). First, sets of co-regulated genes were clustered into modules. Then, singing duration and number of motifs sung were correlated with the gene modules. These modules may consist of genes that are regulated by the same transcription factor (s), genes that regulate the phenotype directly, or genes that are consequences of the phenotype but otherwise unrelated in function to each other. By examining such covariance patterns, the researchers were able to identify two large gene modules that were positively associated with singing and one that was negatively associated. As in previous studies, *FoxP2* mRNA levels were negatively correlated with singing duration and the singing-associated modules. Finally, using a network approach, the authors were able to identify a network of genes that was correlated with *FoxP2* activity. Taken together, this study has provided many novel insights into how the down-regulation of *FoxP2* via singing can give rise to a whole suite of changes in gene expression in a particular brain region (Hilliard et al. 2012).

8.3.3 To Stay or to Disperse?

In landscapes where older populations may go extinct and new populations become established, do dispersal and colonization select upon existing genetic variation? Wheat et al. 2011 used an unusually integrative approach to study dispersal-related life history variation in a meta-population of the Glanville fritillary butterfly (*Melitaea cinxia*). Using microarray analysis, *quantitative PCR*, and physiological measurements in a common garden design, the authors identified metabolic and endocrine factors that may contribute to the disperser and non-disperser phenotype of new and old populations, respectively. Specifically, females from new populations (dispersers) had higher expression of genes involved in egg provisioning in thorax tissue and higher expression of genes involved in maintenance of flight muscle proteins in the thorax than females from established populations (non-dispersers). These findings were complemented with physiological measures, which showed that females from new populations had accelerated egg maturation, higher juvenile hormone titers, and enhanced flight metabolism. By identifying molecular candidate mechanisms of fitness variation maintained by dispersal dynamics in a heterogeneous environment, this study uncovered fascinating and intricate connections between physiology, genomics, ecology and evolution (Wheat et al. 2011).

In addition to genetic variation, other studies have found that neural and genomic plasticity can result in phenotypic variation across generations of butterflies. The spectacular fall and spring migratory patterns of the monarch butterfly (*Danaus plexippus*) provide a compelling example. These migrations span three to four generations because the journey takes longer than the life span of each migrant (Brower 1995). How is it then that they can so accurately navigate the path taken by their ancestors without a single veteran migrant? As migrating butterflies are always on their maiden voyage, a genetic program that integrates two mechanisms in the brain (a molecular clock and a sun compass) provides the basis for the annual migration from Canada to Mexico and back (Reppert et al. 2010). Fall migrant butterflies are reproductively inactive whereas summer monarchs are reproductively active, a switch triggered by juvenile hormone and a cascade of hormon-

ally regulated genes involved in immunity and metabolism. Moreover, microarray analyses have revealed 40 genes that are differentially expressed between summer and fall migrants in relation to migratory behavior (independent of juvenile hormone).

8.3.4 Territorial Defense

Transcriptome studies suggest that the brain can rapidly respond to social stimuli by modulating transcriptional regulatory networks. This type of response requires the interaction between transcription factors and the cis-regulatory sequences of DNA, including promoter and enhancer regions. Bell and colleagues (Sanogo et al. 2012) used a bioinformatics approach to scan the promoters of differentially expressed genes identified in a microarray study that examined the genomic response to territorial intrusion in stickleback (*Gasterosteus aculeatus*). It is important to note that this study did not examine gene expression of the whole brain; rather it examined the transcriptomes of the telencephalon, diencephalon, cerebellum, and brain stem. The researchers found significant correlations between male behavioral response and spatially explicit gene expression patterns in that a large number of differentially expressed genes showed opposite patterns across brain regions. For instance, pro-opiomelanocortin (*pomc*) mRNA was up-regulated in the diencephalon but down-regulated in the telencephalon in response to the intruder. To further explore the mechanisms that could give rise to coordinated change in transcriptional regulatory networks, the authors identified cis-regulatory motifs that were located within 5,000 bp upstream of the differentially expressed genes. This analysis resulted in a list of candidate transcription factors that may be involved in the aggressive response to a behavioral challenge, which can now be used to generate novel hypotheses for future studies into the neurogenomic response to a territorial intrusion (Sanogo et al. 2012). For example, cis-regulatory analysis identified two potential regulators of *pomc* (POU domain, class 3 transcription factor 2 (POU3F2) and the estrogen receptor (ER)), which have previously been shown to regulate *pomc* expression (De Souza et al. 2005). Future studies could employ pharmacological manipulations to determine the functional relevance of ER regulation of *pomc* in the context of territorial defense. Alternatively, one could conduct *chromatin immunoprecipitation sequencing (ChIP-seq)* analysis using an antibody for POUF32 and/or ER to determine on a genomic scale to which extent *pomc* and other genes within the same module are directly regulated by POU3F2 and/or ER.

Songbirds provide another powerful model system to understand the genomics of territorial behavior. For example, male song sparrows of the species *Melospiza melodia* are territorial year-round, yet the neuroendocrine responses to a territorial intruder vary between breeding and non-breeding season (Wingfield and Hahn 1994). Exposure to an intruder in the breeding but not the non-breeding season leads to increases in luteinizing hormone and testosterone. This suggests that the mechanisms that control neuroendocrine responses to social stimuli differ between seasons. In fact, a microarray study by Mukai et al. (2009) demonstrated that an intruder challenge drives differential genomic responses in the hypothalamus depending on season. In autumn and spring, 173 and 67 genes, respectively, were differentially expressed in the control versus territorial intrusion. Because a larger number of genes were differentially expressed between seasons (262), the authors suggested that the underlying seasonal effects on neural gene expression are major contributors to the difference in neuroendocrine responses to social stimuli (Mukai et al. 2009). Overall, these studies show that remarkable genomic plasticity is associated with territorial defense across a broad range of species.

8.3.5 Mating Preferences

Across taxa, variation in the way females choose mates can drive evolutionary change both within and between species. For decades, the research focus has been to identify the male

traits that arouse sexual interest in females (reviewed in Andersson 1994). More recently, however, researchers have begun to identify the physiological and neural processes underlying female choice. The swordtail *Xiphophorus nigrensis*, a poeciliid fish from Mexico, has become one of the most powerful model systems for this kind of research (Houde 1988). In this species, females prefer large males with elaborate sexual traits and courtship behaviors over smaller, more cryptic males that use forced copulation. To investigate the neural and molecular underpinnings that give rise to this preference, Cummings and colleagues (2008) conducted whole brain transcriptome analysis on females given a dichotomous choice between large and small males. What they found was a surprising down-regulation of gene expression when exposed to large males. It is possible that this was the result of a release of transcriptional silencing in response to courtship advances by the males that prepare the female for mating (Wong and Hofmann 2010). Validation experiments using quantitative PCR showed a correlation between individual variation in female preference behavior and the expression levels of several genes, including *neuroserpin*, an extracellular serine protease inhibitor implicated in modulating synaptogenesis and synaptic plasticity (Miranda and Lomas 2006) and exploratory behavior in mice (Madani et al. 2003). However, this study did not examine where in the brain these genes were expressed or how they might differ between closely related species with different *mating systems* (Cummings et al. 2008). To further investigate these findings using in situ hybridization, Wong et al. mapped *neuroserpin* gene expression in female brains, focusing on brain regions of the social behavior network (section 8.5.1, Newman 1999). Quantitative differences in *neuroserpin* gene expression in the *preoptic area* and the medial and lateral zones of the dorsal telencephalon were significantly correlated with female preference behavior (Wong et al. 2012).

In another follow up study, Lynch et al. (2012) compared mate preference behavior between the choosy swordtail females with the Western mosquitofish (*Gambusia affinis*), a poeciliid fish that uses coercive mating tactics. These contrasting behavioral phenotypes provide an excellent comparative model to further investigate the role of *neuroserpin* in mate preference. Using quantitative PCR on whole brain samples, they found that *neuroserpin* levels were positively associated with mate preference behavior in female swordtails but were down-regulated in mosquitofish females expressing male biases. These results suggest that the presence of males in mosquitofish species may inhibit *neuroserpin* expression. Because both gene expression and female behavioral responses to males exhibit opposing patterns between these species, this genetic pathway may potentially act as a substrate for the evolution of mate preference behavior (Lynch et al. 2012). It would be interesting to compare brain region-specific transcriptomes of these females to further investigate the genomic contribution to *neuroserpin*-mediated mate preferences.

8.4 Comparative Approaches

Are there conserved gene modules that are involved in complex social behaviors across distantly related species? Comparative studies that examine closely and distantly related species can provide great insight into the conservation of genome function (O'Connell and Hofmann 2012a). Although striking similarities in neurochemistry and plasticity are seen across wide evolutionary distances, differentiating between conserved and independently evolved traits depends on a well resolved phylogeny with the underlying behavioral mechanisms known for many branches. However, it has been suggested that in cases of behavioral transitions that have occurred independently multiple times (e.g., monogamy), even across large evolutionary distances, similar gene networks have been recruited repeatedly (Toth and Robinson 2007; O'Connell and Hofmann 2011a). Ancestral signaling molecules such as peptide or steroid hormones and biogenic amines likely acted within an ancient neural framework in response to social stimuli (O'Connell and Hofmann 2012a). Over the course of animal evolution,

this simple behavioral framework may have been modified in various ways in order to adapt to new environmental challenges or opportunities that represented rewarding or aversive salience (Barron et al. 2010). In the following section, we will discuss two studies that have compared brain transcriptomes across species in order to gain insight into evolutionary conserved and novel gene expression patterns that are associated with behavioral phenotypes. While there are clearly several obstacles associated with comparative transcriptomics (e.g., increased cost and reliably identifying orthologous genes), this approach promises exciting new insights.

8.4.1 Mating System Evolution

Analysis of gene expression through heterologous hybridization in particular has enabled genome-scale studies in many ecologically and evolutionarily interesting species. Using a cichlid fish microarray platform, Machado et al. (2009) examined neural gene expression levels between individual males and females from a pair of sister species of the Ectodini tribe of Lake Tanganyika cichlids: the polygynous *Enantiopus melanogenys* and the monogamous *Xenotilapia flavipinnis*. Their results indicated that the gene expression profiles are species-specific to a large extent, as relatively few genes show conserved expression patterns associated with either sex. This finding that sex-specific gene expression was highly variable across species indicates that social organization, such as mating system, may play an important role in sculpting transcription profiles in the brain. However, it could also mean that there are core sets of genes whose expression is coordinated across species. Future studies comparing more species will provide us with a better understanding of how these gene sets relate to social phenotypes (Machado et al. 2009).

8.4.2 Evolution of Eusocial Behavior

Comparative genomic analyses can provide great insights into the evolution of mechanisms that regulate social behavior. Toth et al. examined brain gene expression profiles of *Polistes metricus*, a primitive eusocial wasp. Then, the authors compared the results to the database of brain gene expression data for *Apis mellifera*, the advanced eusocial honeybee. To examine genomic variation associated with foraging/provisioning behavior and reproductive status, the authors studied four female wasp groups (foundress, gyne, queen, and worker) using a custom-made *P. metricus* microarray. They found striking differences in the expression across the four groups, many of which showed significant associations with foraging/provisioning status and a handful associated with reproductive status. Next, the authors compared these two differentially expressed gene lists with genes previously shown to be differentially expressed in association with honeybee division of labor and found a striking and significant overlap of genes associated with foraging/provisioning across the two species. Their results suggest that there is indeed common molecular code or a conserved 'genetic toolkit' for division of labor in two independently evolved social insect species (Toth et al. 2010). Future forward and reverse genomic studies that compare distantly related species in a similar behavioral context could provide detailed insights into the mechanisms regulating plastic social behaviors.

8.4.3 Meta-Analyses

While the use of microarray technology may be in decline, this should not stop anyone from analyzing the data collected in these experiments. Meta-analyses of transcriptomic datasets collected within and across institutions can provide a rich source of biological insight when statistical tests are used to rigorously evaluate a single overarching hypothesis.

In Sect. 8.2.1 we already introduced the astonishing life history transitions exhibited by Atlantic salmon (*Salmo salar*), which in their second year of life all females and most males migrate to the sea, where they grow considerably in size before returning to their native stream for reproduction. As discussed above, a subset of

males will remain in freshwater and mature into a small sneaker phenotype (Aubin-Horth et al. 2005). Similarly, some of the migrating fish do not enter the seas directly (early migrants) but instead wait a year before entering the sea (late migrants; Garcia de Leaniz et al. 2007). Immature and sneakers males as well as females differ considerably in brain genes expression profiles (Aubin-Horth et al. 2005). In one of the first meta-analyses of behaviorally relevant transcriptome data, Aubin-Horth et al. (2009) compared the brain expression profiles of all mature phenotypes with that of immature phenotypes and discovered a molecular correspondence between the transition to the sneaker life history in year 1 and the early vs. late migrant transition in year 2. Specifically, these authors discovered a set of 20 genes that are regulated in a concordant fashion in both life history transitions (Aubin-Horth et al. 2009), suggesting that there might be a 'life history transition module' that becomes engaged every time an animal undergoes a major transitions, whether it is in the context of reproduction or migration.

A much more sophisticated meta-analysis was conducted by Ament and colleagues (2012), who developed and applied informatics techniques for discovering meta-associations across transcriptomic experiments collected from many years of research. Deploying these techniques for brain transcriptome profiles from about 400 individual of the relatively docile European honeybee (*Apis mellifera mellifera*) and the more aggressive Africanized honeybee of different ages and worker classes, the authors show that both behavioral/developmental and evolutionary plasticity is regulated by complex interactions between a few common transcription factors, such that distinct combinations of cis-regulatory motifs can give rise to different maturation processes. These findings indicate that phenotypic traits (such as aggression) utilize a common toolkit of regulatory genes, and that variation in the regulatory network can give rise to phenotypic diversity (Ament et al. 2012).

Another fine example of the utility of meta-analysis of large transcriptome datasets comes from the Songbird Neuro-Genomics Initiative (Replogle et al. 2008) where Drnevich et al. (2012) investigated neural gene expression profiles of six different songbird species by analyzing a comprehensive dataset collected by 11 laboratories under a variety of experimental conditions. For example, using the WGCNA approach discussed in Sect. 8.3.2, the authors identified transcriptions factors with high connectivity that may be responsible for coordinating other genes within gene expression modules in area X, a brain region known to be important for song learning. This analysis also found that brain region strongly influenced gene expression patterns, more so than did species (Drnevich et al. 2012). These individual and combined datasets provide a wealth of insights into the relationships between neural anatomy, social behavior in response to environmental cues, and gene expression.

8.5 Reverse Genomics

Like genomic approaches across biology, the field of behavioral genomics has been criticized for its exploratory nature and lack of causality. Given recent advances in next-generation sequencing that allow large amounts of expression and other genome-scale data to be collected at a reasonable expense, it is now high time for researchers in this area to move beyond gene lists and Venn diagrams. The meta-analyses discussed in the previous section provide one promising avenue. But what other approaches could help us to test for function associated with the significant correlations between genomic state and behavior or decision making? Reverse genomic approaches provide a novel and powerful avenue to complement the forward genomic studies discussed above. In order to examine the function of these novel candidates, researchers may choose from a variety of approaches (Fig. 8.1). One option is to manipulate gene expression (e.g., using pharmacology, transgenic techniques, or *siRNA*) and examine the behavioral and genomic consequences of perturbed gene expression. If one is interested in determining the cause

of changes in gene expression, researchers can examine transcription factor binding profiles through ChIP-seq analysis or examination of methylation profiles using *bisulfite sequencing*. These approaches provide insight into whether the observed gene expression changes are due to loss or gain of a *transcription factor binding site* or a change in promoter methylation or histone acetylation, respectively.

Many of the studies discussed in Sects. 8.2–8.4 of this chapter identified interesting and significant correlations between gene networks and a behavioral motif, but only a few have followed up with studies examining the causal or functional relationship. It is worth noting, however, that while we are able to generate lists of sometimes thousands of differentially expressed genes, we can usually experimentally manipulate only a handful of genes, a limitation that requires prioritization of the genes to be manipulated and thus a compelling rationale for selecting such candidate genes in an unbiased manner. In the next section, we will discuss a few studies that have already utilized reverse genomics approaches to better understand the correlations of behavior with one to a few genes identified using forward genomic approaches.

8.5.1 Examining Brain Region-Specific Transcriptomes

It is clear that regions of the brain, having specific biological functions, express a unique suite of genes to perform these functions (Nadler et al. 2006), and in many of the whole- or grossly dissected brain studies described above, lack of spatial resolution was often cited as a reason for not recovering a candidate gene previously associated with the observed behavior or phenotype. This could be because expression of a gene in one brain region can mask its expression in the other regions of the brain. In order to link gene expression to activity within a neural circuit we must look at a higher resolution. Oldham et al. (2006) were among the first to conduct such a spatially explicit analysis. In search for factors that drive evolutionary changes and conservation of gene expression they used a WGCNA approach to compare the gene networks of multiple brain regions (white matter, cerebellum, caudate nucleus, anterior cingulate cortex, and the cortex) in humans and in chimpanzees. The authors noted that genes with high intramodular connectivity were conserved in the human and chimpanzee brain, a finding that supports the idea of conserved molecular mechanisms that govern primate brain organization. Likewise, dramatic differences in gene coexpression networks between the two species are strikingly consistent with the rapid expansion of the cerebral cortex in the lineage leading to humans. By using a comparative approach to examining gene co-expression networks across brain regions, the authors gain valuable insight into how differential network activity in discrete brain regions can be a driver of evolutionary change (Oldham et al. 2006).

Beyond primates, the *dopaminergic reward system* functions to evaluate the salience of a stimulus in the mesolimbic dopamine system, with a key role for dopaminergic projections from the midbrain ventral tegmental area to the regions of the forebrain (Lammel et al. 2011). The social behavior network controls male mating behavior, female sexual behavior, parental behavior, and various forms of aggression. Its involvement in regulating animals' social responses can be understood as a series of hormonally regulated behaviors that are shaped by development, experience and environmental signals (Newman 1999). Together these circuits make up a larger social decision-making network that is highly conserved across vertebrates (O'Connell and Hofmann 2011a, 2012a). Furthermore, this social-decision making network overlaps with what Hoke and Pitts (2012) refer to as the sensory-motor relay, which is important for integrating auditory signals and generating a behavioral output (Hoke and Pitts 2012). While many studies have used immediate-early gene induction to measure neural activity in different social contexts, few have investigated genomic differences across brain regions (Nadler et al. 2006). As methods for whole transcriptome analysis of gene expression from single neurons

or small tissue samples become more reliable (e.g., Morris et al. 2011; Whitaker et al. 2011), we expect to see more studies examining transcriptomic variation within specific neural networks.

8.5.2 Perturbing Molecular Pathways

Many studies have investigated differences between animals displaying varying amounts of aggression (Aubin-Horth et al. 2007; Greenberg et al. 2012; Renn et al. 2008; Sanogo et al. 2012; Toth et al. 2010). These and other studies have implicated a strong role for androgenic and estrogenic regulation of aggressive behavior. O'Connell and Hofmann (2012b) investigated how sex steroids modulate social behaviors, circulating steroids, and the preoptic area transcriptome in dominant and subordinate *A. burtoni* males. They found that social status predicts how sex steroid receptors regulate complex behaviors; androgens and progestins modulated courtship behavior in dominant but not subordinate males, while estrogens modulated aggressive behavior in both dominant and subordinate males. Because of the similar effect of estrogens on aggressive behavior in both phenotypes, the authors then examined the preoptic area transcriptome of estrogen receptor antagonist treated and control treated males. In dominant males, 8.25 % of all genes examined were differentially regulated by treatment while only 0.56 % was differentially expressed in subordinate males. Moreover, the preoptic area transcriptome responses to estrogen receptor perturbation showed very little overlap between dominant and subordinate males. The estrogen receptor was down-regulated in subordinate males, which may have contributed to the lack of gene expression changes associated with the pharmacological manipulation. It seems that inhibition of the estrogen receptor (in combination with other physiological characteristics of subordinate males such as low circulating testosterone levels and the absence of brain activation by the androgen receptor) leads to a remarkable genome-wide suppression of both transcriptional activity and variation in the POA. These results showed for the first time that individuals of the same species can exhibit different behavioral, hormonal, and transcriptomic responses to a perturbation (O'Connell and Hofmann 2012b).

The development of transgenic techniques for the study of behavior in adult animals has and will continue to greatly facilitate our understanding of brain region specific regulation of genes and behavior. Larry Young and colleagues have developed techniques for overexpression of genes in the monogamous prairie vole, *Microtus ochrogaster*, a model system for the study of affiliative behavior (McGraw and Young 2010). Previous studies from the vole community found that the oxytocin receptor expression in the NAcc promoted alloparental behavior and partner preference formation in female prairie voles. Using a viral vector for gene delivery, the researchers found that overexpressing the oxytocin receptor in the NAcc of adult female prairie voles facilitated pair bond formation but had no effect on alloparental behavior. This result demonstrated that oxytocin receptor expression elicited acute activational effects on affiliative behaviors. To examine whether or not it also elicited organizational effects, they used viral vector gene transfer to increase oxytocin receptor density in the NAcc of prepubertal female prairie voles. As adults, these females exhibited both increased alloparental behavior and partner preference. These results are consistent with the hypothesis that oxytocin can have both long-term organizational effects as well as acute activational effects on affiliative behaviors and parental behaviors (Keebaugh and Young 2011). A promising next step would be to compare the transcriptomes of the females.

8.5.3 Functional Genomics Beyond Nucleic Acids

Some of the studies described above identified gene networks that were highly correlated with specific transcription factors. ChIP-seq is an excellent technique for identifying direct targets

of transcription factors to better understand the relationship of these gene networks and their associated behavioral implications (Landt et al. 2012). Work from Eric Nestler's lab and others has found evidence for the role played by several prominent transcription factors, including a Fos family protein (ΔFosB), cAMP response element binding protein (CREB), and nuclear factor kappa B (NFκB), among several others, in the brain reward circuitry (Nestler 2012b). By integrating data from behavioral assays and DNA expression arrays with detailed analysis of chromatin remodeling and histone modification at drug-regulated gene promoters, these researchers were able to identify genes that are regulated by drugs of abuse via the induction of ΔFosB. These findings established that chromatin remodeling can play an important regulatory role underlying drug-induced behavioral plasticity and provided novel insight into the mechanisms by which ΔFosB regulated expression of specific target genes in reward pathways and contributes to addiction (Nestler 2008). Likewise, the study by Ament and colleagues discussed above found associations between behavior and the transcription factors *Creb*, *br*, *dl*, *Xbp1*, and others, suggesting that these genes are particularly promising candidates for functional characterization in future experiments (Ament et al. 2012). While these approaches have become feasible even in non-traditional model systems, few studies use ChIP-seq in behaviorally relevant contexts. It is clear, however, that future experiments should further investigate the interactions between transcription factors and DNA.

8.6 Into the Future

Research into the functional neurogenomics of social behavior has given us great insights into the evolutionarily conserved and plastic mechanisms that modulate neural and molecular responses to changes in an animal's social environment. We want to review and briefly summarize the major insights we have gained over the past decade and then discuss where we think the field might be heading.

8.6.1 Emerging Themes of Behavioral Genomics

What are some of the general insights that have emerged from the more than a decade of research behavioral genomics? First, we now know that the genome can change much more rapidly and dramatically in response to environmental stimuli than anyone thought possible (e.g., ca. 10 % of protein coding genes in only 30 min; Cummings et al. 2008). These dynamic properties likely reflect the real-time adjustments in the activity of gene networks in response to – and in preparation for – changes in the activity of both neural circuits and neuroendocrine systems (Hofmann 2010). Furthermore, a large fraction of the genome is involved in these responses, not merely a few genes (Renn et al. 2008; Whitfield et al. 2003). Particular functional groups or gene families appear to be involved in different kinds of plastic phenotypes as suggested by Aubin-Horth et al. (2009) and Sanogo et al. (2012). It also appears that a small set of transcription factors governs global changes in response to different environmental or social stimuli, giving rise to co-regulated gene sets or modules (Ament et al. 2012). Importantly, gene expression profiles can vary considerably across brain regions (Oldham et al. 2006), underscoring the importance of examining individual brain nuclei or even single neurons in future studies. Finally, there is increasing evidence that conserved or deeply homologous gene modules can be associated with behavioral phenotypes that have evolved independently (O'Connell and Hofmann 2012a; Toth and Robinson 2007). No one could have predicted any of these surprising and fundamental insights during the early days of behavioral genomics, but we believe that the best is yet to come.

8.6.2 New Horizons

With the rapid advances in sequencing technology, RNA-seq, ChIP-seq and related technologies are poised to replace microarray-based approaches for functional analyses of the dynamic genome. For example, a recent

review by Hitzemann et al. (2013) illustrates why RNA-seq is a superior strategy. While microarray analysis of gene expression is a mature technology, is relatively inexpensive, and has well developed analysis pipelines, it is limited by the need for primary sequence information and poor detection of rare transcripts, allelic variation, and splice variants. RNA-seq on the other hand requires no prior knowledge of expected transcripts, has wide dynamic range of detection, and provides information on individual sequence variation. However, it is still more expensive, requires bioinformatic expertise and high performance computing infrastructure (Hitzemann et al. 2013).

Both microarray and RNA-seq technology face similar difficulties when it comes to comparative studies across distantly related species. Hofmann and colleagues demonstrated the feasibility of heterologous hybridization for comparative analysis of gene expression. In the experiments, only genes with minimal sequence divergence could be compared (Renn et al. 2004). The same will probably be true for RNA-seq since part of the pipeline requires that orthologs be called, but this technology will have as added benefit information on sequence variation. Along those same lines, the choice of reference genome will be an important decision to make for comparative studies. One can use a well annotated genome from a more or less distantly related species or one can assemble reference transcriptomes *de novo* from the data collected. In any case, researcher need to keep in mind that the method chosen can have profound impacts on the outcome of the analysis (Grabherr et al. 2011).

Many of the studies described above obtained correlational results that suggested that, for example, a given transcription factor or set of transcription factors might be responsible for regulating dramatic genomic changes in response to stimuli (e.g., Ament et al. 2012; Nestler 2008; Sanogo et al. 2012). A number of techniques are available for testing the functional implications of such inferences. One option is to manipulate gene expression (e.g., using pharmacology, transgenic techniques, or siRNA) and examine the consequences of perturbed gene expression at the level of both behavior and transcriptome. If antibodies of the candidate transcription factor are available, one could use ChIP followed by PCR or *deep sequencing* to identify its direct targets. Also, several techniques for characterizing the response on a more spatially refined level are available, which allows the analysis of gene expression changes within and across the nodes of a neural circuit implicated in behavioral regulation. This list of reverse genomic approaches is by no means exhaustive, rather it is meant to raise awareness that methods well established in other fields (such as genetics, neuroscience, or microbiology) can be applied to of the integrative study of behavior and evolutionary and ecological genomics in general.

As more and more transcriptional datasets are made publically available, we are confident that these big data sets will be harnessed for biological discovery and that new approaches will be developed that will facilitate the comparison of data collected on different platforms (both existing and those yet to be invented). In conclusion, we urge researchers in the area of ecological and evolutionary functional genomics to combine forward genomics approaches (i.e., from phenotype to behaviorally relevant gene modules) with reverse genomic approaches (i.e., manipulating of novel gene modules to examine effects on behavior, hormones, and the genome itself). With such an integrative approach we will gain fundamentally new insights into the relationship between gene expression and behavior and their evolution. We can gain a lot of novel and fundamental insights into behavioral plasticity by examining genome activity across brain regions and discovering whether variation in gene expression profiles is due to differential regulation of chromatin structure and/or transcription factors. The future of this endeavor is sure to yield many great discoveries.

Acknowledgments Research in our lab has been supported by NSF, NIH-NIGMS, and the Alfred P. Sloan Foundation. We want to thank the editors for giving us the opportunity to review the state of our field; Jeff Gross, Suzy Renn, and members of the Matz Lab and the Hofmann Lab for discussion; and Ryan Wong and two anonymous reviewers for helpful comments on earlier versions of this chapter.

Glossary

Bisulfite sequencing The use of a bisulfite treatment of DNA followed by deep sequencing to determine the methylation pattern.

Chromatin immunoprecipitation sequencing (ChIP-seq) The use of high-throughput sequencing technologies to sequence the regions of the genome that interact with a given protein of interest, often a transcription factor.

Deep sequencing The process of obtaining both the sequence and frequency of RNA or DNA molecules in a given tissue at a given time through any number of next-generation sequencing technologies.

Dopaminergic reward processing The role that dopamine plays in the integration of environmental and physiological cues and the encoding of the rewarding properties of a stimulus to generate an adaptive behavioral response.

Gene network A statistical representation of correlated gene expression data for identifying sets of co-regulated genes or gene modules.

Gene module A set of co-regulated genes.

Immediate early genes (IEGs) Genes, usually encoding transcription factors, that are rapidly and transiently activated in response to a wide variety of cellular and extracellular stimuli.

Mating system A classification of the time, place, and number of partners an individual has during reproduction.

Microarray An array of thousands of RNA, cDNA, or DNA probes, usually printed on a glass slide with which the activity of thousands of genes can be assayed simultaneously.

Next-generation (NextGen) Sequencing (also referred to as high-throughput sequencing) Any of a number of technologies that yield millions of sequences concurrently by parallelizing the sequencing process, thereby significantly lowering the cost of sequencing while increasing the amount of data.

Nucleus accumbens (NAcc) A mesolimbic brain region that receives massive dopaminergic input from the VTA and is intimately involved in evaluating stimulus salience and reward processing.

Preoptic area (POA) A region of the forebrain that is important for regulating many social behaviors in males and females as well as other basic physiological functions such as energy homeostasis and thermoregulation.

Quantitative PCR (qPCR) A molecular technique used to amplify and simultaneously quantify a targeted DNA or RNA molecule.

Reproductive tactic Behavioral strategy used by individuals to increase their reproductive success.

RNA sequencing (RNA-seq) The use of high-throughput sequencing for quantitative analysis of short cDNA reads.

Small interfering RNA (siRNA) A class of double stranded RNA molecules, usually 20–25 base pairs, that interferes with the expression of genes with complementary sequence.

Social dominance High status or hierarchical rank in a social group.

Striato-pallidal Area X A region of the songbird brain that has been linked to singing. It is part of the basal ganglia, a set of nuclei that have been widely implicated in motor control and learning.

Transcription factor binding site Short stretches of DNA where other molecules, specifically transcription factors that regulate gene activity, can bind.

Transcriptome The set of all the expressed RNA molecules (or a subset, e.g., mRNA) in a given tissue or cell.

Ventral tegmental area (VTA) A region of the brain that is major source of dopamine in the brain. It plays an important role in evaluating the salience of environmental stimuli and signaling motivational events.

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