Functional Genomics of Neural and Behavioral Plasticity

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ABSTRACT: How does the environment, particularly the social environment, influence brain and behavior and what are the underlying physiologic, molecular, and genetic mechanisms? Adaptations of brain and behavior to changes in the social or physical environment are common in the animal world, either as short-term (i.e., modulatory) or as long-term modifications (e.g., via gene expression changes) in behavioral or physiologic properties. The study of the mechanisms and constraints underlying these dynamic changes requires model systems that offer plastic phenotypes as well as a sufficient level of quantifiable behavioral complexity while being accessible at the physiological and molecular level. In this article, I explore how the new field of functional genomics can contribute to an understanding of the complex relationship between genome and environment that results in highly plastic phenotypes. This approach will lead to the discovery of genes under environmental control and provide the basis for the study of the interrelationship between an individual's gene expression profile and its social phenotype in a given environmental context. J Neurobiol 54: 272–282, 2003

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INTRODUCTION

How nature and nurture contribute to who we are, what we do, and why we do it, has long been debated by scientists and philosophers alike. We now know that there are biologic roots to our behavior. In fact, behavioral genetics has provided us with a wealth of information regarding the roles of individual genes in the implementation of behavior. Yet behavior (just like any other phenotypic trait) is commonly influenced by both environmental and epigenetic factors (see Pigliucci, 2001). Because of this intricate relationship between genes and environment, we can distinguish four major challenges facing the genetic study of behavior that have to be addressed before we will gain a better understanding of the genetic mechanisms underlying behavior.

(1) First, the genetics of behavior (and of most common diseases) requires the dissection and analysis of complex phenotypes or traits. Complex traits are determined by many factors (genetic, epigenetic, and environmental) whose interactions are nonlinear and often unpredictable. As a consequence, the genetic architecture of a complex trait cannot be derived from the individual effects of each of the component factors alone, however well studied they are. It is in principle possible to define the genetic components of a complex trait in terms of Mendelian segregation and location along a genetic map. It is, however, crucial to recognize that the genetic architecture is not so much a fundamental biological attribute of a trait as it is a characteristic of a trait in a particular population dependent on gene and genotype frequencies, the distributions of environmental factors, age and sex, etc.

For example, in typical, late-onset Parkinson Disease, a neurodegenerative disorder that is associated with a reduction of dopaminergic activity in the substantia nigra, tracking down disease-causing genes has been elusive. However, the existence of "susceptibility" genes has been suggested (Martin et al., 2001), particularly in the context of the alleged disease-causing role of environmental toxins (Checkoway and Nelson, 1999). Several twin studies have so

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far been unable to identify genetic factors (Ward et al., 1983; Tanner et al., 1999). Surprisingly, patients with Parkinson often have peculiar premorbid personality traits (e.g., industriousness, punctuality, lack of novelty seeking, low life-time risk for cigarette smoking) whose relevance for the disease onset is entirely unclear (Menza, 2000).

Clearly, if many genes contribute to a given complex phenotype, it can become difficult to dissect its genetic basis, even when a few candidate genes have been identified (Tabor et al., 2002). Although one particular gene may contribute significantly to the trait (within a given genetic and environmental background), one often can only estimate how many other loci are involved. To solve this problem, researchers have developed a range of strategies, the most important of which is the mapping of quantitative trait loci (QTL) to defined chromosomal locations along the genome (see Flint, this issue). However, it is often challenging to make the connection between a given marker (that represents a QTL on the genomic map) and the actual gene (or group of genes) that participate in the quantitative trait under study.

(2) Another problem facing the genetics of complex traits arises due to the fact that the contribution of genes may be epistatic rather than additive (Wade, 2001; Wade et al., 2001). Behavioral geneticists encounter epistasis (gene–gene interactions) most often as differences in phenotype that relate to the genetic background or "modifier genes" of the animal strain used (Gerlai, 2001; Nadeau, 2001). These gene interactions are nonlinear and intricate, which greatly complicates the identification and mapping of genes underlying a trait. Similarly, many gene-targeting studies (where one particular gene was "knocked out" in a transgenic animal) have shown that phenotypes can differ greatly depending on genetic background and "compensatory mechanisms" (Gerlai, 2001).

(3) The relationship between genotype and environment (G $\times$ E) presents a third problem confounding the study of complex behavior. In most behavioral genetic experiments environmental conditions are kept constant (for a discussion of standardization see Würbel, 2002; van der Staay and Steckler, 2002). However, it has become increasingly clear that complex and nonlinear G $\times$ E interactions exist (Pigliucci, 2001; Sokolowski & Wahlsten, 2001; Wade, 2001). A striking example of the complex interplay between genotype and social environment has been presented recently. Caspi et al. (2002) showed in a human population that a polymorphism in the promoter region of the monoamine oxidase A gene is correlated with violent behavior in adult males, but only in men who were maltreated as children.

In a different study, Sillaber et al. (2002) showed that mice lacking the corticotropin-releasing hormone receptor (CRH1-R) show enhanced and persistent alcohol consumption (compared with wild-type controls) only after stressful experiences such as social defeat. Also in mice, social stress exacerbates stroke outcome by suppressing expression of Bcl-2, a proto-oncogene that promotes cell survival and protects against cell-death (De Vries et al., 2001).

Finally, the increase in mortality (mostly through coronary heart disease) in Eastern Europe after the dissolution of the Soviet Union is a sobering example of G $\times$ E interactions. Psychosocial stressors as a consequence of societal uncertainty are thought to be the primary cause of this unexpected change (Stone, 2000).

Many studies have correlated differences in behavioral phenotypes with differences in the nervous system. Often, the conclusion has been that the neural differences are causally responsible for the behavioral differences observed. For example, LeVay (1991) reported in a widely cited article that the volume of a nucleus in the anterior hypothalamus was larger in heterosexual than homosexual men. Many interpreted this result as evidence that differences in the brain caused the observed differences in sexual behavior, possibly reflecting the influence of genetic factors during development. However, until cause and effect have been identified, the alternative explanation that these differences were a consequence of years of differential sexual behavior is just as valid. In this context it is interesting to note that in adult male rats differences in sexual experience lead to differences in motor neuron size (Breedlove, 1997). It is important to keep in mind that both genotype and environment contribute to the phenotype.

(4) Finally, means of inheritance exist that are not dependent on DNA. During gametogenesis, epigenetic modifications of the genome occur (i.e., genomic imprinting), which can lead to profound behavioral differences in the offspring (Li et al., 1999; Keverne, 2001). Furthermore, maternal factors in the egg (Davdison, 1986), maternal care (Meaney, 2001), as well as traditions (mediated by social learning; Avital and Jablonka, 2000) can contribute to the transmission of neural and behavioral phenotypes. As a consequence, a purely gene-based approach to the dissection of complex phenotypes may overlook important factors of inheritance for a given trait.

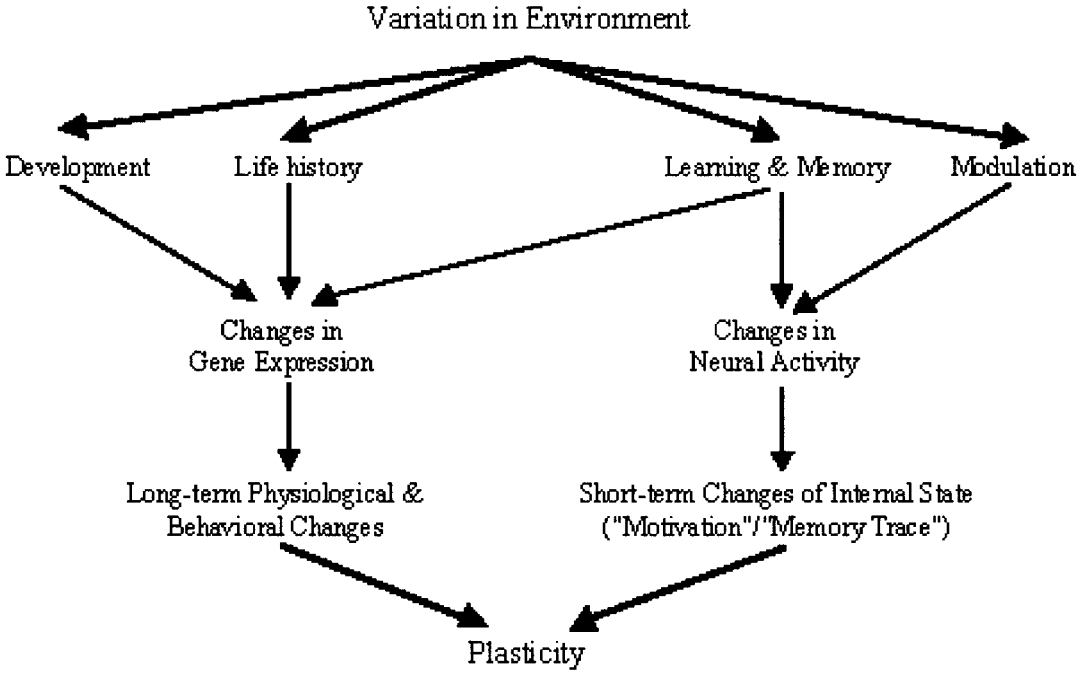

Figure 1 A concept map of phenotypic plasticity as it applies to brain and behavior. See explanation in text.

FUNCTIONAL GENOMICS OF NEURAL AND BEHAVIORAL PLASTICITY

In this essay I will introduce a conceptual framework that aims to elucidate neural and behavioral plasticity by applying a functional genomics approach. I believe that this approach is complementary to the approaches conventionally used in behavioral genetics, and that it may help to overcome some of the shortcomings discussed above.

The analysis begins at the level of the phenotype by describing and analyzing the neural, endocrine, and behavioral phenotypes that an organism can potentially realize depending on environmental conditions. By integrating concepts from neurobiology, ethology, and evolutionary biology with powerful genomic technologies this approach will facilitate a more comprehensive understanding of the roles that genes and environment play in the dynamic and plastic sculpting of brain and behavior throughout life.

Plasticity in the nervous system comprises the functional and structural changes in information processing after the initial formation of neuronal contacts. This is obviously a very general definition, which nevertheless has proven to be useful, and has allowed us to gain insights into many plastic processes in the brain. Figure 1 illustrates the different processes and time scales where plasticity can be adaptive. In cases where the frequency of the environmental change (the stimulus) is high or when immediate action is required, nervous systems can respond quickly by means of modulation or through learning. Those dynamic changes of the internal state of an animal (e.g., motivation, memory trace) are usually achieved, at least initially, by changes in neural activity and excitability or by endocrine responses. Short-term changes will then often lead to subsequent changes in brain and behavior (e.g., memory formation) through differential gene expression as well as structural and physiologic changes. Environmental stimuli that are slow or relatively infrequent can alter developmental trajectories and shift neural functioning throughout life history, even in adult animals (e.g., seasonal and use-dependent changes).

CICHLID FISHES AS A MODEL SYSTEM FOR THE STUDY OF PLASTICITY

It is important to note that plasticity as it relates to changes in the nervous system (and, subsequently, behavior) after a change in the (social) environment is only a subset of phenotypic plasticity. Over the last

decade, the study of adaptive phenotypic plasticity has become a major area of research within evolutionary biology (Schlichting and Pigliucci, 1998; Pigliucci, 2001). Phenotypic plasticity is seen as a reflection of the reaction norm (as determined by the genotype) that buffers the organism against fluctuations in the environment. Changes in morphology, physiology, and behavior as they relate to survival and reproductive fitness have long been studied within the context of life history theory (Stearns, 1992) and the tradeoffs have been analyzed in much detail in a few select model systems (Zera and Harshman, 2001). As an example, consider the differential allocation of resources towards growth and reproduction in the African cichlid fish *Astatotilapia (Haplochromis) burtoni* (Hofmann et al., 1999): Reproductively active territorial males spend all their time and energy on territory maintenance and mate attraction (as well as sperm production), whereas nonreproductive males spend most of their time feeding and, as a result, show increased growth. Once a subordinate animal has grown sufficiently to challenge a territory owner, a rank reversal accompanied by a change in phenotype often occurs, as size is an important predictor of dominance.

Other cichlid species show a comparable phenotypic diversity, although the cellular and physiologic mechanisms have yet to be studied in detail. It is believed that the extreme morphologic as well as behavioral plasticity exhibited by species from this family of fishes have contributed to the astonishing radiations of species with varied feeding types and diverse social phenotypes (Barlow, 2000). Their remarkable plasticity both within and across closely related species makes cichlids an ideal model for the comparative study of complex and plastic behaviors.

GENE EXPRESSION PROFILING IN THE BRAIN

The basic idea of the functional genomics approach to phenotypic plasticity is to monitor the activity of thousands of genes simultaneously in a particular tissue or brain area while the organism is undergoing environmentally or developmentally induced plastic change. Differentially expressed genes are then hypothesized to be involved, either directly or indirectly, in the implementation of the respective phenotype.

The (nearly) completed sequencing of several animal genomes (human, mouse, pufferfish, zebrafish, fruitfly, nematode) has provided us with a mountain of information that we have just begun to mine for biologic meaning. Highly parallel high-throughput technologies have become available that allow the monitoring of the activity of thousands of genes simultaneously by measuring their mRNA abundance. Examples include the PCR-based total gene expression analysis (TOGA, Sutcliffe et al., 2000) and serial analysis of gene expression (SAGE, Velculescu et al., 1995). The most prominent gene expression profiling technique, however, utilizes DNA arrays, either as macroarrays where cDNAs are spotted onto nylon membranes (Barrett et al., 2001) or microarrays. In the latter case, oligonucleotides or (annotated) cDNAs representing known genes and splice variants are spotted in high density onto glass microscope slides. Alternatively, microarrays can consist of thousands of cDNAs of unknown sequence derived for instance from a cDNA library. This way microarrays can be constructed for tissues where only few expressed genes are known or for organisms whose genomes have not yet been sequenced. cDNAs that are found to be differentially present in a given experiment can then later be accessed in the clone library and sequenced. These sequences can often be tentatively assigned a particular function based on sequence similarity as determined by BLAST in Genbank and annotated genomic databases for particular organisms. Although less expensive in terms of material costs and time, anonymous arrays provide a lot less information than annotated arrays.

Once candidate genes have been identified, they can guide the discovery of the genetic pathways and interactions relevant for the implementation or maintenance of a given phenotype. The potentially most useful genes in the context of behavior are those that have been shown to play a role in neurotransmission and/or neuroendocrine systems (Pfaff, 2001). For example, a preliminary screen that compared the preoptic areas of territorial and nonterritorial *A. burtoni* cichlids yielded 59 differentially expressed genes, among them neurotransmitter receptors, neuropeptides, ion channels, growth factors, and protein kinases (Hofmann et al., 2001).

Applying genomics in neuroscience brings its own challenges as well as opportunities (Cao and Dulac, 2001; Luo and Geschwind, 2001; Nisenbaum, 2002). One obvious problem with gene expression profiling in the nervous system is that because of the vast diversity of neuronal cell types differences in biologically meaningful messages may go undetected because of their relatively low abundance. This problem can in principle be solved by extracting RNA from smaller, that is, more defined, areas of the brain until one finally harvests only single cells (e.g., brain punch

microsampling: Holter et al, 2001; laser capture microdissection: Scheidl et al., 2002; single-cell PCR: Eberwine et al., 1992). However, these solutions result in very small amounts of mRNA. To overcome this problem without pooling of tissue samples while maintaining a reliable representation of transcriptional complexity, several RNA amplification protocols have been developed (linear: Wang et al., 2000; Baugh et al., 2001; PCR-based: Dulac and Axel, 1995). Once sufficient amounts of RNA derived from two different experimental conditions are available, the resulting probes are labeled with two different fluorescent dyes (usually the cyanines Cy3 and Cy5) and used to target the array in a competitive manner. An interesting new approach to consistent harvesting of neural tissues across many animals was developed by Brown et al. (2002), and is based on the idea of using so-called "voxels," that is, normalized cubes of brain tissues as source of the RNA. If dissection is done carefully, transcription profiling can also provide a molecular correlate for previously described brain regions, thus providing an extension of neuroanatomy (Zhao et al, 2001).

It has become clear that repeated sampling, normalization, and thorough statistical analysis, often combined with modeling, are necessary to derive meaningful insights from microarray experiments (Novak et al, 2002). I do not have the space to discuss the important problems associated with the analysis of microarray data (but see Quackenbush, 2001; Tseng et al., 2001; Nadon and Shoemaker, 2002). It should suffice to mention that the field is now moving away from the initial naïve use of fold changes in gene expression to more sophisticated statistical analyses (e.g., Aach and Church, 2001; Kim et al., 2001). Hierarchical clustering algorithms have become the *de facto* standard to uncover patterns as well as to facilitate comparisons across experiments (Eisen et al., 1998).

Validation of microarray results is often required before any biologic conclusion can be drawn because false positives can mislead the investigator. Useful validation techniques are Northern blot analysis and increasingly quantitative real-time PCR (Rajeevan et al., 2001). *In situ* hybridization is a particularly important tool for confirming the localization of differentially represented mRNAs in the brain (Nisenbaum, 2002), and successful attempts of high-throughput automation have been made with the GenePaint system developed by Tecan in Switzerland (URL: http://www.tecan.com/).

Clearly, expression profiling is the important first step towards a genome-based analysis of brain and behavior. However, one should keep in mind that mRNA levels are not necessarily representative of protein levels and/or activity. Although protein arrays may be available in the future, proteomics in neuroscience is just beginning (Grant and Blackstock, 2001), and at this point no such techniques are on hand for large-scale high-throughput analysis of proteins in the brain. For now, if we want to ascertain that mRNA differences are representative of differences at the protein level we will have to use conventional techniques such as immuncocytochemistry, ELISA, etc.

The wealth of data generated by a microarray can be overwhelming. Bioinformatics tools are necessary not only to handle and analyze the data, but also to be able to interpret them. Fortunately, there are efforts underway to implement a standardized set of rules for performing as well as reporting microarray experiments (the MIAME standards: Brazma et al., 2001; Geschwind, 2001), which will facilitate both exchange and (meta-) analyses of large data sets. However, this will not be enough: imagine a particular set of genes that may become upregulated when the synapses of a cell undergo plastic change during development and/or learning. Are genes that have been implicated in synaptic remodeling differentially regulated? Are other coregulated genes members of the same "synaptic remodeling pathway" but have not yet been described in this context? How do we decide which genes are the most promising to follow up on once we have determined that they are differentially expressed in our experimental system? Currently, all we can do is use our expertise and experience, do some background reading, and essentially answer all these questions "by hand."Because so much information is generated by microarray experiments, it becomes clear that some standardized and computerized way of data analysis and biologic interpretation will become necessary.

Microbiologists have implemented pathway databases containing detailed information on metabolic pathways for microorganisms (Kanehisa et al., 2002; Karp et al., 2001). They also have developed tools that enable the mapping of microarray data onto these databases. These tools enable the user to determine instantly which pathways (and which of their genes) were affected by a particular experimental treatment (Dahlquist et al., 2002; Grosu et al., 2002). Such gene expression maps can be used as a gene discovery tool to identify coregulated genes or to uncover previously unknown genetic functions. Within the neurosciences, efforts to include neurobiologic data and knowledge in Web-based databases have recently picked up

speed, mostly within the functional imaging community (Toga, 2002). However, to my knowledge, there is no comprehensive searchable database dedicated to molecular, physiologic, and anatomical pathways of brain function.

Microarray technology is not limited to expression profiling. For example, in so-called ChIP[2] or ChIP array experiments, genomic DNA that has been enriched for areas of active transcription in the genome by chromatin immunoprecipitation (ChIP) with an antibody that recognizes DNA binding proteins can be hybridized to a microarray containing intergenic regions (Ren et al., 2000). This approach facilitates the discovery and analysis of previously unknown regulatory regions (Liu et al., 2002). Analysis of regulation of genes and gene networks appear to be as important for the understanding of regulation as the examination of the coding regions of individual genes.

REGULATION

The idea that changes in gene regulatory regions have been a major driving force in the course of evolution has first been put forward by Britten and Davidson (1971). Subsequently, King and Wilson (1975) proposed that mutations in regulatory sequences account for the major biologic differences between species based on the observation that the small genetic differences alone cannot explain the huge morphologic and behavioral differences that distinguish humans from chimpanzees. Recently, Enard et al. (2002) have reported significant differences between these two species in the expression profiles of several different tissues. Consequently, spatial and temporal differences in gene expression both during development and in the adult must play an important role. Although there has been tremendous progress in our understanding of the evolutionary implications of transcription regulation during development (see Chapter 7 in Carroll et al., 2001), we still know very little as to how transcriptional regulation in the nervous system produces different physiologic and behavioral outcomes. In a recent essay, Baker et al. (2001) propose on the basis of work done in fruitflies that dedicated regulatory genes like *fru* "build" regulatory circuits in the brain that are committed to specific complex behaviors. Obviously, these behaviors and the underlying circuits can be modified by experience. I believe that a thorough comparison of expression profiles will provide us with insights into the genetic architectures that implement alternative phenotypes. It is still early days, but there are already some fascinating results.

In cichlid fishes, for example, it is believed that visually guided mate choice behavior is one of the major forces driving the explosive radiation of this group (Seehausen et al., 1997). At the ontogenetic level, Kröger et al. (2001) have shown that exposure to different visual environments modifies visual processing in the cichlid retina. In addition, Carleton and Kocher (2001) were able to show for three species of Lake Malawi cichlids that their respective cone opsin genes code for nearly identical visual pigments. Thus, mutations in the coding regions cannot explain the observed differences in spectral sensitivity between these species. As these authors showed by means of quantitative real-time PCR, the precise regulation of relative expression levels of individual cone opsin genes leads to sensory differences in the visual system. They conclude that behaviorally and ecologically relevant "variations in cichlid spectral sensitivity have arisen through evolution of gene regulation, rather than through changes in opsin amino acid sequence."

Based on these insights I would like to speculate that in cichlids, where plasticity is widespread and has frequently been argued to be a major source of adaptive radiation in this group, the spatial and temporal orchestration of a limited number of transcription factors is used to implement different social phenotypes. To illuminate this idea, imagine a contest where the participants are all equipped with an identical set of plastic building blocks. The task is to build a vehicle that can move a distance between two points in space. A number of solutions will be proposed (e.g., automobile, bicycle, airplane, boat) that will all do the job albeit with slight differences in performance. Now suppose we select for speed and against airborne vehicles: the bicycle and airplane would quickly become "extinct," while automobile and boat may race head to head. However, each contestant could quickly rebuild her plastic-block model (within certain limits or constraints) to accommodate current selection pressures. Figure 2 shows a more realistic example where using the exact same building blocks a fish exhibiting a red face and yellow stripes can "switch colors." Interpreted in biologic terms, subtle regulatory responses to environmental changes that an organism experiences may result in major changes in gene expression and their downstream phenotypes. For example, in *A. burtoni*, upregulation of hypothalamic gonadotropin releasing hormone expression is a consequence of social dominance and is followed by a complex cascade of downstream events that ulti-

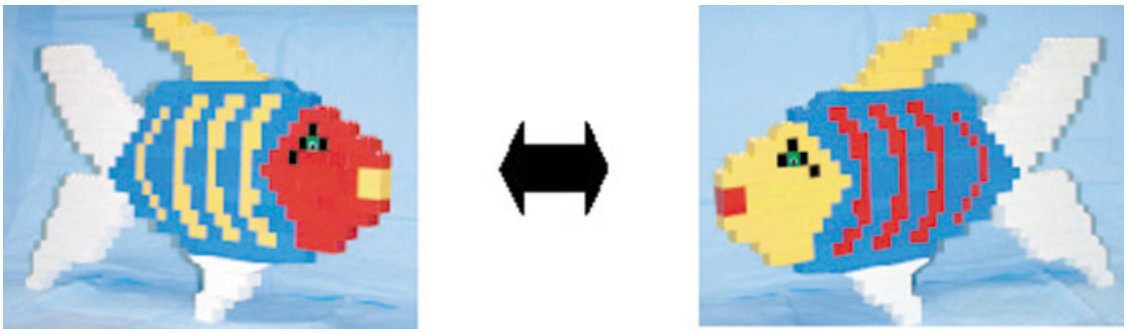

Figure 2 Example of a "phenotypically plastic" fish, built with plastic building blocks, to illustrate how differential regulation of gene expression can implement plastic changes in phenotype.

mately result in rapid sexual maturation (Francis et al., 1993; White et al., 2002). By the same token, as indicated by the work of Carleton and Kocher (2001), small changes in regulatory regions may have similar effects over evolutionary time (which runs fast in cichlids), thus providing a basis for the evolution of novelties and ultimately speciation (Agrawal, 2001).

PLASTICITY AND ROBUSTNESS

The plastic building block metaphor exposes two problems, however: first, living organisms will always be subject to constraints that limit their plasticity, both at the ontogenetic and evolutionary level. Second, to respond to selection pressure, the plastic building block player needs to rebuild the model from scratch, which is biologically often not feasible. Interestingly, this realization leads to a question that has rarely been asked by neuroscientists and organismal biologists alike: how does an organism that is undergoing plastic (and often drastic) change avoid disintegration? How is it that an animal can change from one phenotype into the other while still being "functional," for example, able to forage and escape from predators? Many animals have solved this problem by introducing a developmental stage that allows an almost complete rebuilding of the organism while maintaining its integrity (e.g., the pupa in holometabolous insects; Truman and Riddiford, 2002). But such a drastic reduction in functionality may not be adaptive for animals that change their phenotype as adults. Figure 3 illustrates the problem with an example from *A. burtoni*. Only 3 of the 14 phenotypic characters known to be under environmental (social) control are shown. Note that the (reversible) changes are in the endocrine axes (reproduction, growth) and in behavior asymmetrical depending on the direction of the phenotypic change, likely displaying an "environmental optimism" in an unstable world where reproductive opportunities may change quickly (Hofmann and Fernald, 2001; White et al., 2002). Moreover, time constants and direction of change in growth and reproduction, which are usually tightly coupled in the homeostatic animal, are different during phenotypic transition. In my mind, plasticity is only half understood until we begin to address the question of robustness. Although there have been some studies on how robustness emerges in genetic and cellular networks (Barkai and Leibler, 1997; Alon et al., 1999), the question has received only scant attention from neuroscientists. The interplay between plasticity and robustness in the nervous system has been highlighted in an exemplary fashion in marsupials where the removal of up to 75% of the cortical neuroepithelial sheet early in development results in normal relationships between visual, somatosensory, and auditory cortical fields on the remaining cortical sheet (Huffman et al., 1999; Krubitzer and Huffman, 2000).

Careful perturbations of the environment can not only be used to study the molecular mechanisms that enable a system to buffer its phenotype *against* environmental change. In fact, I believe that thoroughly designed expression profiling experiments can also help to unravel the mechanisms constituting robustness during plastic change. For example, we are currently comparing expression profiles of territorial and nonterritorial fish (which represent the two opposing phenotypic endpoints in this system) as well as profiles taken at selected time points during transition from one phenotype to the other. This way, we may be able to identify genes that show a characteristic activity pattern only in the transition phase. One challenge will be to determine whether any of these genes are "robustness genes" that encode, for example, specific transcription factors, which actively stabilize the

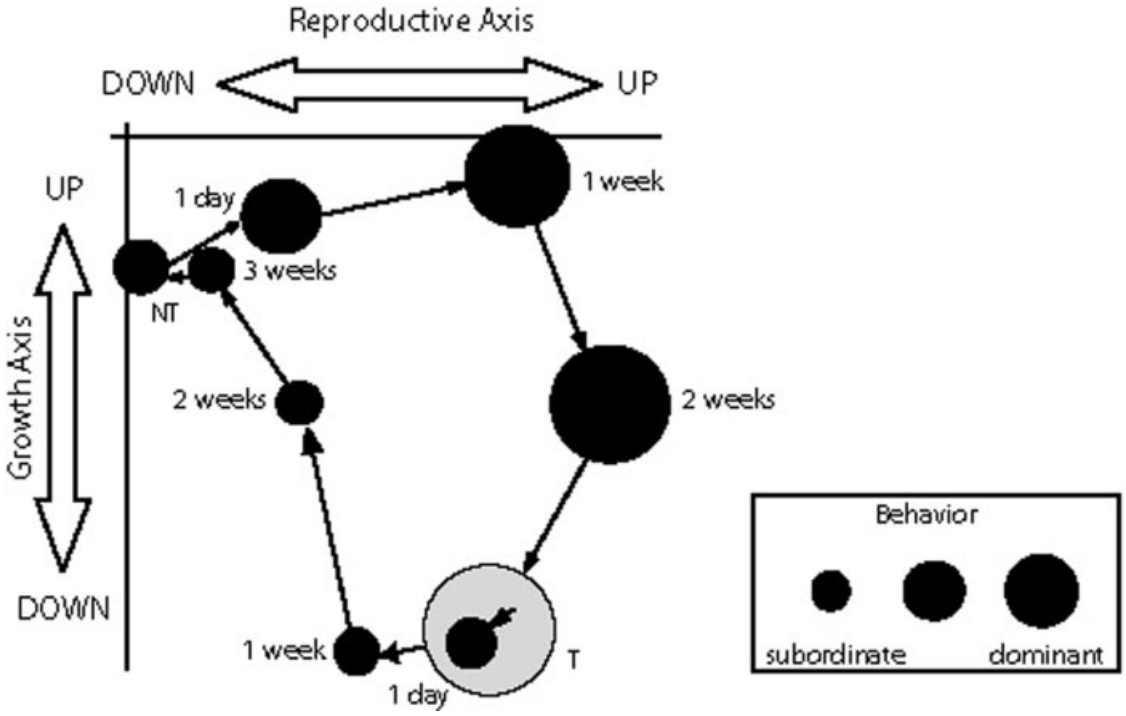

Figure 3 Behavioral and neuroendocrine trajectory of phenotypic plasticity in the African cichlid fish *A. burtoni*. Changes of reproductive and growth axes plotted as dominance behavior (circles) changes with time. Note the hysteresis-like function as changes in social phenotype are asymmetric with regard to behavior and endocrine status: Although the behavioral change after social defeat (T→NT) is immediate and faster than in ascending animals (NT→T), the latter gain fully reproductive physiology (T) within only a week, while T→NTs allow up to 3 weeks for their reproductive axis to be downregulated to a reproductively inactive (NT) level. The growth axis remains upregulated while the reproductive axis becomes activated as an animal ascends in the dominance hierarchy. However, once established as a dominant and reproductively active animal (T), growth is downregulated. Conversely, after losing dominance (T→NT) and attaining nonreproductive (NT) status, growth becomes upregulated again at the same time as reproduction is downregulated. Note that the temporal dynamics of only three of about 15 phenotypic characters known to be under social control in this species are shown. (Modified after Hofmann and Fernald, 2000, 2001; White et al., 2002.)

organism during change. Alternatively, differential expression of these genes could be merely a consequence of the transition itself. Alternatively (or in addition), robustness may emerge from the action of complex networks of molecules, neurons, and circuits.

CONCLUSIONS

The ideas presented here are an attempt to combine mechanistic and organismal concepts with modern genomics techniques to gain an integrated understanding of behavioral and neural plasticity. Looking at one gene or one phenotypic character at a time cannot address the complexity of gene–environment interactions underlying plastic phenotypes. My hope is that by applying functional genomics to study the molecular, neural, and neuroendocrine basis of plasticity and its twin sister, robustness, we will gain insights that will ultimately unite reductionist and integrative approaches to brain and behavior.

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