Commentary: With Gottlieb beyond Gottlieb

Ehud Lamm

Introduction

Technological and methodological advances, in particular next-generation sequencing and chromatin profiling, has led to a deluge of data on epigenetic mechanisms and processes. Epigenetic regulation in the brain is no exception. Extending our existing frameworks for thinking about psychobiological development to include molecular epigenetic mechanisms is a worthy and timely goal. This is what Vanessa Lux attempts in her article.

Making sense of the new data requires new conceptual tools. Two distinct and clearly interrelated frameworks are needed: a developmental one, centered on plasticity and robustness, and an evolutionary one, focused on adaptation and trans-generational time-scales. The former should clearly address the role of experience in brain and behavioral development as well as of the role of behavior in influencing the experiences of the organism. This reciprocal or "cybernetic" role of development, as Conrad Waddington called it, has significant evolutionary consequences as also recognized by Gilbert Gottlieb and other thinkers who studied the interaction of development and evolution.

Development, in the abstract

Alas, there is no abstract framework for thinking about development. This judgment may seem overly harsh, so I should clarify what it is that I claim is missing. Consider, as an analogy, the conceptual framework of Neo-Darwinism. Any Neo-Darwinian explanation of adaptation must make use of notions such as selection pressure, genotypes and phenotypes. Genotypes and phenotypes are properties of evolutionary individuals which comprise populations. The evolutionary process affects the frequencies of these properties in populations, based on universal properties of genetic inheritance. This explanatory scheme provides a lot of wiggle room: fitness and selection pressures may be multiply realized, as indeed can inheritance. Various properties of genotypes, such as chromosomal linkage and epistasis, may be part of the explanation or not, depending on the question at hand. Evolutionary individuals may be organisms, groups, and so no. While each of these topics can be a can of worms, the general

framework abstracts away from the details. No account of development plays a similar role. However, such an account is needed if general claims about development as such are to be made. Such an account would be tremendously helpful when we try to combine an evolutionary account with a developmental one – the ultimate goal of Evo-Devo. Of course it is entirely possible that such a general account of development is an unattainable, elusive, goal.

By way of illustration, here are a few alternative frameworks for thinking about development (this is not a comprehensive list).

- The Epigenetic Landscape (Conrad Waddington): Waddington's informal model emphasized the role of interaction with the environment and the importance of canalization. The genomic system was depicted as unaffected by development, an external constraint.
- Probabilistic Epigenesis (Gilbert Gottlieb): Development is conceptualized as a hierarchy of bi-directional interactions and explicitly includes the effect of experience on gene regulation. Genes themselves are unaffected by development, an external constraint.
- Gene Regulatory Networks and related models (e.g., Eric Davidson's GRNs): Here the logic of gene regulation is the focus. Development is conceptualized as mostly flowing from the genes up. Interactions between levels of organization are not emphasized. Evolution predominately involves changes in regulatory elements that control gene action rather than in functional genes.
- Dynamic Patterning Modules (Stuart Newman): Major aspects of morphological organization are explained as an emergent result of physical processes affecting cell aggregates. A small developmental-genetic toolkit supports wide phenotypic plasticity that does not depend on selection or genetic change.
- Bernard Machines (J. Scott Turner): Development is the result of physiological interactions of many homeostasis preserving entities that are referred to as "Bernard Machines". The internal environments they create, termed persistors, are longer-lived than the machines that maintain them, possibly extending beyond organisms and outliving them. Persistors interact bi-directionally with the environment and with the genes in a goal-directed and responsive manner.

How do development and evolution affect each other? According to several of these accounts, evolutionary change in genes is typically a late stage in the entrenchment of evolutionary change. It is preceded by developmental and behavioral adjustments by the organism in response to novel circumstances. These change the selection pressures experienced by the organism thereby affecting genetic evolution. One interesting possible outcome is genetic assimilation, a process in which a behavior that is acquired or learned becomes automatic and innate to a certain degree, allowing individuals to reduce the effort and resources required to learn it and freeing up resources for new learning. This results

from traditional natural selection operating on variations in learning ability and predispositions. An additional way of making developmental results more robust is to establish various physical or social scaffolds that support and direct development by ensuring that organisms have the appropriate experiences at appropriate times. This can be achieved by niche construction. Social scaffolding of development and cultural niche construction are critical in the evolution and development of cognition.

Probabilistic Epigenesis seems on the face of it particularly well-suited for the addition of epigenetic mechanisms. Epigenetic mechanisms may however have significant implications on how development and evolution interact, as I discuss below. It thus makes sense to consider the evolutionary consequences of Probabilistic Epigenesis and how the model is transformed when evolution is made an explicit part of it. A second worry is that, unlike some other frameworks, Gottlieb's model is non-mechanistic: It specifies levels of organization and their interaction but is for the most part silent about mechanisms. Adding epigenetic mechanisms immediately raises the question why not include myriad other mechanisms operating at each level of the developmental hierarchy?

The Neuronal Epigenome

Epigenetic mechanisms are mechanisms that affect gene regulation and regulate genome dynamics. The conformation of chromatin, the three dimensional physical genome comprised of DNA and proteins, can change in development as a result of epigenetic modifications and thereby affect gene expression. The chromatin possibly also serves as a platform along which epigenetic signals propagate adding to the importance of genome organization. Two major categories of epigenetic marks in the genome are DNA methylation and histone modifications. A third category of epigenetic processes, the most famous of which is RNAi, a post-transcription silencing mechanism, involve non-coding RNAs. Processes of the three types interact in complex ways, many of which are not yet fully understood. Epigenetic changes can be transient but can also be stable for long periods of time.

Defects in epigenetic mechanisms lead to mental illnesses (Telese et al. 2013). For example, mutations in the DNA methylation "reader" MeCP2 enzyme have been implicated in Rett Syndrome which manifests in learning, behavior, and language deficiencies. Another striking example is the Angelman and Prader-Willi syndromes that result when genomic imprinting control regions are not properly methylated. Individuals with these syndromes exhibit severe mental retardation.

In a recent review of the role of epigenetic mechanisms in learning and memory Blaze and Roth (2013) conclude that "epigenetic modifications subserve information storage in the CNS in order to modify subsequent behavior." Telese et al. (2013) note that "Accumulating lines of evidence have shown that crucial players

of synaptic plasticity function as epigenetic regulators and that mutations and variations in their genes are linked to mental illnesses." Moreover, epigenetic changes in the brain, in particular changes in DNA methylation, are activity-dependent and may possibly regulate gene expression in response to neuronal activity (Telese et al. 2013). The bi-directionality of control this suggests directly supports Gottlieb's Probabilistic Epigenesis.

Another important discovery is that psychological stress can causes epigenetic changes, in particular methylation changes in the hippocampus. From a developmental perspective, it is significant that early traumatic experiences such as infant separation and experience with abusive care-takers lead to a pattern of long-lasting epigenetic changes in multiple brain regions. These affect stress responsivity, among other things. Rewarding experiences, such as a high-fat diet, can likewise cause persistent epigenetic changes in reward mechanisms. Remarkably, maternal diet during pregnancy can affect epigenetic marks in the reward circuitry of offspring (reviewed in Blaze and Roth 2013). Both these results indicate how acquired epigenetic changes can be inherited in animals with germline-soma separation: in utero experience can affect offspring; and parental behavior, such as neglect, caused in part by epigenetic modifications in the parent, can cause offspring to reproduce a similar pattern of epigenetic marks. Blaze and Roth suggest that epigenetic mechanisms may thus allow the transgenerational transmission of experience-driven gene changes and phenotypes. This goes beyond Gottlieb and the extension proposed by Lux. It has also been suggested that early in the evolution of associative learning the neurohormonal outcomes accompanying and following stress-related associative learning triggered not only changes in nerve cells, but also changes in the germline (Ginsburg and Jablonka 2010).

The effects of stress on the neuronal epigenome match the more general observation that stress of various kinds produces genome-wide epigenetic changes in many taxa. Either directly or as a result of epigenetic changes, stress can also cause genetic changes, i.e. changes in the DNA sequence. Transposable elements that are normally silenced may become activated in stress conditions, increasing genetic variation (reviewed in Lamm and Jablonka 2008). Furthermore, it has been suggested that by utilizing epigenetic variations (e.g., ncRNAs) organisms may be able to evolve adjustments to development more quickly than by modifying transcription programs controlled by transcription factors that are utilized extensively in the genome.

Extending Probabilistic Epigenesis

In her article, Vanessa Lux suggests augmenting Probabilistic Epigenesis by incorporating molecular epigenetic mechanisms as mechanisms that mediate between neurons' genetic activity and their neuronal activity and the neuronal activity of their surrounding cells. As the brief discussion above shows this

is indeed a plausible place to locate some epigenetic activity. However, this move raises some questions. Why add just epigenetics and not all regulation and stabilization mechanisms (e.g., transcription factors)? Gottlieb did not make these explicit in the model. Why are genetic changes (e.g., transposition), not included in addition to changes in genetic activity? Stress conditions can affect both. Finally, is the model supposed to be mechanistic or just highlight equifinality and the bi-directional, multi-level, nature of development? Many kinds of mechanisms operate at each of the levels. Some mechanisms, such as social institutions that provide developmental scaffolding and cultural niche construction, may be of particular interest for thinking about human evolution and cognitive development yet they were not included by Gottlieb in the canonical figures illustrating Probabilistic Epigenesis, even though the effects of social interaction were central to his work.

Following her modification of Gottlieb's model, Lux classifies molecular epigenetic mechanisms in neurons into what she calls three functional contexts: the genomic, the developmental, and the synaptic, thereby distinguishing between the multiple functions a single molecular mechanism can serve. Genomic functions involve genomic repair mechanisms and maintenance of low expression of pro-apoptotic genes. Developmental functions involve the differentiation of neuronal stem cells. Synaptic functions involve synaptic plasticity and function (e.g. memory formation). The molecular mechanisms Lux discusses are, strictly speaking, all genomic mechanisms that operate at the genomic level and potentially affect transcription and chromatin dynamics. Lux's classification is meant to aid hypothesizing about the interaction of the various functions of epigenetic mechanisms in psychobiological development and seems like a useful heuristic. It highlights the role played by epigenetic mechanisms in inter-level interactions. However, Lux omits from the classification the hereditary function that epigenetic modifications may possibly have. This adds a fourth functional context for epigenetic mechanisms: mediating between experience and behavior, in one generation, and genetic activity, in the next. Lux's model suggests the following additional questions:

1) Are there other levels in the brain, above the synaptic level, where molecular epigenetic mechanisms play a role?

2) Is the modified model too restrictive compared to Gottlieb's model and applicable only to a subset of psychobiological development rather than development in general? It is plausible that experience can affect development through regulatory changes, and epigenetic changes in particular, in nerve cells outside the brain and in cells other the neurons (e.g., hormone producing cells). To what extent are such effects relevant to subsequent psychobiological development?

3) If, indeed, all of the epigenetic mechanisms discussed are strictly speaking genomic, is the idealization made by the revised model not misleading?

Perhaps genetic and genomic levels should be distinguished as two separate levels of organization.

4) Is the proposed classification, that is derived from the interactions that make up Gottlieb's graphical model, specific to psychobiological development or should it generalize to other cells? In other words, should similar classifications of molecular epigenetic mechanisms by produced for other types of cells?

Conclusion

Gilbert Gottlieb's Probabilistic Epigenesis emphasized two crucial aspects of psychobiological development and evolution: that development does not proceed from the inside out but is constantly affected and directed by experience, and that evolutionary change need not, and typically does not, originate from a genetic mutation. Rather, organisms adjust to their environment or seek new opportunities, thereby changing the context in which selection then occurs. What role, if any, do molecular epigenetic mechanisms that operate at the level of the genome have in these two processes?

The first process, the developmental, can comprise changes at various levels of organization, clearly including changes in brain circuitry and synaptic strength. Some changes involve gene regulation where epigenetic processes of methylation and histone modification obviously play a role. The evolutionary process, however, may not seem directly affected by molecular epigenetic mechanisms operating in somatic cells, though other epigenetic inheritance processes may significantly affect behavioral and brain evolution (Jablonka and Lamb 2005). This conclusion however may be too hasty. At the heart of the integration of evolution and developmental plasticity are processes and mechanisms involved in adjusting to novel challenges. Here, epigenetic mechanisms often have a role, as a variety of studies of large epigenomic changes in stress conditions indicate. The role of epigenetic mechanisms in learning is likewise significant. Epigenetic changes can reduce and channel variability, as Lux's suggests, but they can also produce variability. Searching the genomic space and searching the synaptic space may both turn out to depend on the action of epigenetic mechanisms. Feedback, stablization, and backup mechanisms ensuring overall robustness of the developmental process are a necessary background for search processes and here again epigenetic mechanisms may have an important role.

The genome, understood as the chromatin and all related mechanisms, should be understood as a developmental system in its own right. The DNA sequence, epigenome, transcriptome, and proteome are all interacting, and the conformation of the structured, physical, genome changes developmentally, affected by and affecting epigenetic mechanisms. Gene expression, construed in the traditional way, is one manifestation of these processes (see Lamm 2011). Lux suggests a useful heuristic classification of the functions played by epigenetic mechanisms in

psychobiological development in the brain and justifiably highlights their role as mediators between levels, emphasizing the role of feedback loops in development. Like Gottlieb and Waddington before him, however, she takes genes for granted. As an idealization the proposal makes a lot of sense. Ultimately, a developmental picture of the genome will be needed, one in which epigenetic mechanisms will play a significant role. In such a framework it is unlikely that their genomic functions could be divorced from their developmental function.

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