Timing neurogenesis: a clock or an algorithm?

Julien Pigeon, Bassem A Hassan

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Julien Pigeon[1] and Bassem A. Hassan[1]*

[1] Institut du Cerveau-Paris Brain Institute (ICM), Sorbonne Université, Inserm, CNRS, Hôpital Pitié-Salpêtrière, Paris, France.

*Corresponding author. Email: bassem.hassan@icm-institute.org

Abstract

Emerging evidence supports the existence of dedicated molecular mechanisms under evolutionary selection to control time during neurogenesis. Here, we briefly review these mechanisms and discuss a potentially useful conceptual framework inspired by Computer Science to think about how these biological mechanisms operate during brain development and evolution.

Main text

In a fundamental sense, time represents the continuous progression of existence and events. It unfolds moment by moment and permits a structured chronology where events occur in a specific sequence, not randomly or all at once. This framework allows events to be ordered from the past through the present into the future, which in turn helps us understand causal chains where causes precede effects.

In developmental biology, the concept of time is closely intertwined with the pace of embryonic development. This connection can be viewed in at least two ways: the specific sequence of events that occur in a particular chronological order as well as the total amount of time this sequence occupies. Both of these dimensions are found in one of the most studied and evolutionarily conserved biological mechanisms, neurogenesis, the generation of neurons from neural progenitor cells (NPCs). Not only are different neuronal subtypes generated at different time points in a specific order, but the total amount of time dedicated to neurogenesis is itself a species-feature of brain development. Intriguingly, while the sequential stages of brain development share ancestral blueprints, the temporal dynamics can

diverge considerably among species. The human brain, for example, undergoes a particularly extended period of neurogenesis compared to rodents and even other primates, allowing for a disproportional increase in the number of neurons, especially in the cerebral neocortex [1], [2]. Thus, there is a correlation between differences in timing and brain size, presumably intimately linked to differences in cognitive capabilities.

This review will discuss the temporal dynamics of neurogenesis and the factors that drive neural progenitor cells (NPCs) to their transition into post-mitotic neurons. We will explore a conceptual framework for understanding how and why changes in timing arose during evolution and what this framework predicts as possible future experimental avenues.

- **Time in cell state transitions and its control through intrinsic and extrinsic cues**

Classical analyses using immunohistochemistry and, more recently, single-cell transcriptomics have revealed that distinct cellular states are associated with specific gene expression patterns. Therefore, the acquisition of a specific cellular identity requires a remodeling of these gene regulatory networks (GRNs) that underlie cell state-specific expression patterns. Studies across species and neural tissue types, from fruit flies to human *in vitro* models, have shown that the commitment of a NPC to neurogenesis is influenced by internal and external cellular cues that act on its GRN to determine whether it will continue to self-renew as a progenitor or differentiate into a neuron. One of the causal molecular mechanisms of this dynamic equilibrium between the progenitor and neuron states is mediated by feedback regulation between the Notch signaling pathway and a class of basic Helix-Loop-Helix (bHLH) transcription factors called proneural proteins [3]. When the proneural protein is maintained and stabilized in a NPC, it will overcome the anti-differentiation signal of the Notch pathway and promote neurogenesis ([3]–[5]). Additionally, in the local environment of these NPCs, many secreted morphogens by specific brain regions and the NPCs themselves with their neuronal progeny will weigh on this balance. For example, WNT, FGF, and Shh, play a significant role in maintaining progenitors' self-renewal through different processes to repress proneural genes (reviewed in [6]). In other words, controlling the temporal dynamics of the expression and activity of transcription factors by intrinsic and extrinsic cues can significantly impact the timing of the switch from progenitor self-renewal to differentiation and, thus, the time available for neurogenesis. In the case of the particularly slow pace of human cortical neurogenesis, it has been shown that a human-specific variant of the Notch protein contributes to the developmental tempo by

intrinsically promoting increased levels of Notch activity in progenitors, presumably inhibiting proneural protein expression [7**]. On the other hand, the conserved Amyloid Precursor Protein involved in Alzheimer's disease is required in human cortical NPCs to mediate extrinsic WNT signaling to delay the NPC to neuron transition, and this correlates with reduced expression of the proneural protein Neuorgenin2 [8**]. Finally, evidence shows that the rate of degradation of transcription factors involved in neurogenesis is slower in humans than in mouse NPCs [9**]. There is also evidence that more stable or longer-lived proneural proteins correlate with [10] and can cause [11*] an increase in neuronal production. Emerging evidence also causally implicates mitochondrial metabolism in the NPC-neuron fate decision [12]. Whether this is mechanistically linked to changes in transcription factor activity or stability remains unclear. Interestingly, however, stem cell programming into neurons by Neurogenin2 overexpression results in large-scale remodeling of cellular organelles and proteomes, suggesting that a link may also exist during normal neurogenesis [13*].

Overall, these examples support the view that the temporal dynamics of neurogenesis is controlled mainly by cell-intrinsic mechanisms that appear to converge on regulating the expression and activity levels of transcription factors and that these mechanisms operate on different time scales in different species. However, this only pushes the question from why the phenotypes (i.e. neurogenesis) emerge on different time scales to why the intrinsic cellular mechanisms operate on different time scales. This question is particularly intriguing because the operant mechanisms discovered so far are highly conserved across eukaryotes. Although mitochondrial homeostasis and metabolism or protein turnover seem to be slower in humans than in mice, for example, there is no evidence that the highly conserved enzymes that carry out these reactions have intrinsically slower substrate processing rates. Finally, given that all these mechanisms appear to converge on the activity of transcription factors that are also themselves highly conserved, the conundrum seems even more perplexing. One way to think about the problem from a broader perspective is to shift from a mechanistic view to a theoretical view in order to provide a general framework into which the currently understood mechanisms would fit. This would guide future hypotheses that could be tested with mechanistic approaches. What might such a theoretical framework look like?

Time from Computer Science to Biology

Perhaps our human conception of time as something to be measured with clocks misleads our understanding of developmental time. In Computer Science, time is a measure of computational complexity. It describes the number of elementary operations an algorithm uses to process a set of input data. Interestingly, this measurement, known as time complexity, characterizes how the execution time of an algorithm increases as a function of the size of the input. The larger the input data set, the longer an algorithm will take to complete its processing.

To translate this concept to biology, one needs to begin by defining what constitutes an algorithm and what constitutes the source of data. We propose that an operationally useful way of thinking about this is to consider the sum of all cellular biological reactions as the algorithm. After all, during development, various proteins literally "process" input in the shape of other biomolecules or metabolites to produce a cell state transition as a phenotypic output. More importantly, the genome is the only source of biological data for which we have evidence supporting a causal role in generating evolutionary change. As we have argued above, and as decades of molecular genetic mechanistic studies have shown, the biochemistry of fundamental cellular processes, that is to say, the algorithm in this metaphor, is highly conserved. What is changing is the data in the shape of evolutionary innovations in the content of genomic information. A particularly striking example in the context of neurogenesis and neuronal differentiation comes from the transplantation of human cortical pyramidal neurons into the neonatal mouse brain, which develops much faster than the human brain. What these studies show is that the maturation of the human neurons in the mouse brain takes up to a year [14], [15] suggesting that the mechanisms underlying the developmental timing are cell intrinsic and encoded by the human genome. A last important angle to consider is the processing modality of the cellular algorithm. Most, if not all, cellular interaction networks are partially promiscuous. This is referred to as "many-to-many" interaction networks, and they are very prevalent in ligand-receptor and transcription factor dimerization networks, for example, including for key neurogenesis pathways such as WNT, Notch and the proneural proteins. It has recently been argued that such networks are both more robust and have greater computational power [16*] than highly specific, one-to-one interaction networks, likely explaining why they have been favored by evolution. Furthermore, just like for an algorithm, several biological processes run in parallel but converge on a terminal step that induces the transition to the next state, such as proneural protein levels and activity in NPCs (see next paragraph). This terminal step acts as a coincidence detecting gatekeeper ensuring

that different aspects of cellular metabolism are all compatible with the impeding state transition.

In summary, a potentially helpful way to think about how the temporal dynamics of biological processes operate is to assume that a highly conserved algorithm of flexible biochemical interactions processes a variable amount of data encoded by the genome. In this conception, the differences in developmental timing would arise principally from differences in the amount of information encoded by the genome.

- **Sources of differences in the amount of genomic information**

One important source of increase in genomic information during evolution is gene duplication, a major evolutionary driving force [17]. Interestingly, in *Drosophila*, where proneural genes were discovered, there are 58 bHLH genes whereas there are 39 in *C. elegans* and 125 in humans [18], [19]. Moreover, these transcription factors induce the expression of Notch ligands such as Delta and Serrate in flies. Here again, we observe an evolutionary change in the number of proteins. In flies, there are two ligands of Notch, Delta and Serrate, while in vertebrates there are 5 known ligands: Delta like DLL1, DLL3, DLL4 and the Jagged family composed of Jagged1 and Jagged2. In addition, the number of Notch receptors has been multiplied. In flies, there is a single Notch receptor, while in humans, there are 4 (Notch1-4) [20]. These ligands and receptors are expressed in complex overlapping spatio-temporal patterns. Altogether, these duplications of many genes multiply the possible interactions between ligands and receptors. The increased complexity in these ligand-receptor interactions carrying both activation and inhibition signals can create delays in signal interpretation into a specific pattern of effector activation [21]. Furthermore, different ligands can transmit opposing patenting information [22] and thus their co-expression would also require more time for that information to be resolved into a fate decision. Finally, many molecular interactions, especially ligand-receptor interactions and gene activation by proneural proteins, are quantitative in that they depend on the absolute amount of active protein. Because levels of any biomolecule at any time are subject to variation due to both stochastic and random noise, the greater the number of proteins in an interaction network, the more noise in the network and, thus, the longer it may take for efficient signal transmission [23]. Having a single Notch receptor and a single ligand makes the signal transmission direct.

As discussed above, gene duplication in humans appears to have been taken to a new level with entire families of rapidly evolving genes dedicated to the attenuation of neurogenic signals, as in the example of Noch2NL [24**].

Other potential sources of increase in information are gene regulatory elements, notably highly conserved enhancer/repressor sequences that have been subject to variation only in the human lineage called Human Accelerated Regions (HARs). Interestingly, more than half of these regions tested in vitro displayed enhancer activity, specifically in NPCs [25]–[27] such as HAR5 that enhances WNT signaling through FZD8 to promote NPCs self-renewal [28]. Another source of quantitative regulation of protein levels is post-transcriptional control by noncoding RNAs, considered as another driver of brain evolution. They represent 10 to 15% of the human genome [29]. There is almost twice the number of miRNAs in humans than in mice and six times that in *Drosophila* [30]. For example, the miR-2115, a great ape-specific miRNA, promotes NPCs proliferation by targeting the ancient gene ORC4 mutations which cause the Meier-Gorlin microcephalic syndrome [31*], [32]. Furthermore, a primate lncRNA mediates Notch signaling during neuronal development by sequestering miRNA, promoting NPCs self-renewal [33]. The increase in the number of quantitative post-transcriptional inhibitors of neurogenic proteins would be expected to delay the accumulation of these proteins to functional levels and thus delay cell state transitions that depend on these proteins.

In summary, the combination of gene duplication and increase in the number of regulators increases both the total amount of information the cellular algorithm must processes as well as the amount of noise in this information. The combined effect is to lengthen processing time and thus delay the time interval of cell state transitions during neurogenesis.

- **Time, robustness, and size**

Why would natural selection have favored these temporal delays over evolutionary time, in the primate and specifically human lineage for example? Needless to say, evolution does not have a plan to produce humans. It must, therefore, be that such a delay in neurogenesis confers a selective advantage for the fitness of the organism. One argument for which there is both experimental and theoretical evidence is that the increase in the number of genes, the promiscuity of protein interactions, and the stochastic noise in molecular processes all confer greater robustness on cell state transitions and developmental patterning [16*], [23] in this scenario, the principal selection pressure is on robustness and the increase in neurogenic time

is an unintended but inevitable consequence; an exaptation [34] in the "Gouldian" sense, rather than an adaptation. However, it can also be argued that increasing the amount of time a neural progenitor remains in a progenitor state increases the number of neurons it can generate, and this, in turn, results in increasing the brain's adaptative cognitive capabilities. In this scenario, the random accident of gene duplication, for example, gave rise to time delay, which gave rise to an increase in brain size with increased cognitive capabilities, and it is this that confers the selective advantage [35]. The truth most probably is that both views are correct. Exaptation and adaptation are synergetic, and both processes can be seen at work at organismal, cellular, and subcellular levels in evolutionary history [36].

Conclusion

In this review, we hypothesized that the amount, rather than the type, of information encoded by the genome controls developmental time, with a focus on neurogenesis and its mechanisms as an example. Using a concept borrowed from computer science, we suggest that fundamental cellular metabolism, the *type* of information in this metaphor, constitutes a conserved algorithm that processes information at a conserved pace. In contrast, the total amount of molecules and their interactions constitute the data that this algorithm must process, and the *amount* of this data dictates the time necessary for a developmental process like neurogenesis to be completed. The emerging evidence that conserved processes and genes can have different effects on neurogenesis in different species [8**], [9**] by altering time is testament to the key importance of temporal regulation in brain evolution. If the genomic changes that drive brain evolution have the main effect of altering developmental time, it can be reasonably argued that temporal patterning is the main feature of the genetic code, and it is related to the total amount of "data" encoded by the genome. To test this idea, future experiments and modeling efforts should be directed towards greater accuracy in the measurement of time in molecular processes at high resolution during cell state transitions and deciphering the causal effects of quantitative changes in protein levels on the timing, size and complexity of neurogenic processes.

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J.P. and B.A.H conceived of and wrote the manuscript. J.P. came up with the idea of comparing biological processes to an algorithm.

Competing interest:

The authors declare no competing interests.

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