

Stranger in a Strange Land: Using Heterotopic Transplantations to Study Nature vs Nurture in Brain Development

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Journal of Experimental Neuroscience
Volume 12: 1–4
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DOI: 10.1177/1179069518758656



ABSTRACT: The mammalian brain develops from a simple sheet of neuroepithelial cells into an incredibly complex structure containing billions of neurons with trillions of synapses. Understanding how intrinsic genetic programs interact with environmental cues to generate neuronal diversity and proper connectivity is one of the most daunting challenges in developmental biology. We recently explored this issue in forebrain GABAergic inhibitory interneurons, an extremely diverse population of neurons that are classified into distinct subtypes based on morphology, neurochemical markers, and electrophysiological properties. Immature interneurons were harvested from one brain region and transplanted into a different region, allowing us to assess how challenging cells in a new environment affected their fate. Do these grafted cells adopt characteristics of the host environment or retain features from the donor environment? We found that the proportion of interneuron subgroups is determined by the host region, but some interneuron subtypes maintain features attributable to the donor environment. In this commentary, I expound on potential mechanisms that could underlie these observations and explore the implications of these findings in a greater context of developmental neuroscience.

KEYWORDS: Interneurons, development, transplantation, migration, parvalbumin, somatostatin, neuronal nitric oxide synthase

RECEIVED: January 19, 2018. **ACCEPTED:** January 21, 2018.

TYPE: Commentary

FUNDING: The author(s) disclosed receipt of the following financial support for the research, authorship, and/or publication of this article: This study is supported by the NICHD intramural research program.

DECLARATION OF CONFLICTING INTERESTS: The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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COMMENT ON: Quattrococo G, Fishell G, Petros TJ. Heterotopic Transplantations Reveal Environmental Influences on Interneuron Diversity and Maturation. *Cell Rep.* 2017 Oct 17;21(3):721-731. doi: 10.1016/j.celrep.2017.09.075. PubMed PMID: 29045839; PubMed Central PMCID: PMC5662128.

Commentary

We recently published a paper in *Cell Reports* exploring the role that the environment plays in the fate decisions and maturation of interneurons.¹ The motivation behind this research is grounded in the long-studied question in developmental biology: What characteristics of a cell are predetermined via intrinsic genetic programming and which features are driven by environmental interactions? While *Drosophila* neuroblast differentiation is primarily driven by intrinsic temporal patterning,² there is a rich literature in mammalian neurogenesis highlighting the importance of environmental cues in modulating cell fate. Deciphering this “nature vs nurture” relationship becomes even more complex when studying the developing brain, with its abundance of different cell types, connectivity patterns, and environmental niches. GABAergic inhibitory interneurons are an incredibly diverse cell population that can be classified into dozens of subtypes based on morphology, connectivity, neurochemical markers, and electrophysiological properties. Thus, interneurons are simultaneously both an optimal and challenging experimental paradigm to explore how the interplay between genetic programs and environmental factors determines cell fate and maturation.

Nearly all forebrain interneurons originate from several transient brain structures in the embryonic brain, the medial ganglionic eminence and the caudal ganglionic eminence (MGE and CGE, respectively). The MGE and CGE give rise to nonoverlapping interneuron subtypes that migrate

throughout the forebrain and terminate in a variety of brain regions. Evidence from many labs indicates that initial fate decisions occur around the time of cell cycle exit within the MGE and CGE. Several factors play important roles in regulating the initial fate decisions of these progenitors, such as their spatial location, temporal birthdates, and the mode of neurogenic divisions.^{3–7} However, the extent to which most interneuron characteristics (location, mature markers, morphology, physiological properties, etc) are preprogrammed or determined by environmental interactions is unknown.

We approached this project with multiple candidate mechanisms to explain the mature distribution of interneuron subtypes, with the assumption that different interneuron features could be generated from alternate or multiple mechanisms. One hypothesis is that interneurons are initially fated into “cardinal classes” (eg, somatostatin- [SST⁺] or parvalbumin-expressing [PV⁺]) during embryogenesis, and then interaction with the proper brain environment drives “definitive specification” into more specialized subtypes (eg, PV⁺ basket or chandelier cells)⁸ (Figure 1). This appealing hypothesis proposes a gradual differentiation process that is initiated embryonically and refined throughout development. Although this general concept is likely true for certain interneuron characteristics such as morphology and connectivity, more recent evidence supports the idea that specific interneuron subtypes can be genetically defined much earlier during embryogenesis.^{9–11} In



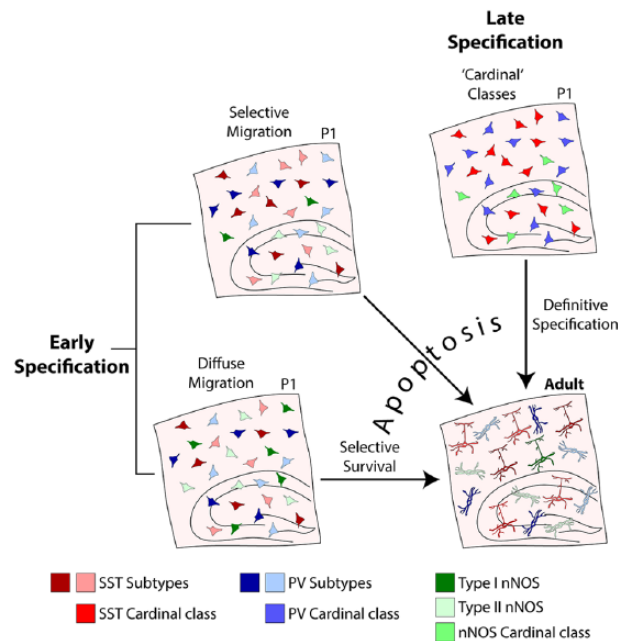


Figure 1. Potential mechanisms to generate the spatial distribution of interneuron subtypes. To generate the mature distribution pattern of interneurons, distinct interneuron subtypes could be defined early during embryogenesis or postnatally after cells have migrated to their proper brain regions. If interneuron subtypes are defined early (as most evidence seems to support), then the proper spatial distribution could be obtained via “selective migration” to specific brain regions (*top left*) or diffuse migration followed by “selective survival” (or selective apoptotic cell death, *bottom left*). Alternatively, immature precursors could be initially fated into general cardinal classes (SST⁺, PV⁺, and nNOS⁺) and then undergo more definitive specification into specific subtypes on maturation with their environment (*top right*). Note the differential localization of nNOS⁺ cells (and type I/type II subtypes) between the cortex and the hippocampus. nNOS indicates neuronal nitric oxide synthase; SST, somatostatin; PV, parvalbumin.

this case, early defined interneuron subtypes could undergo selective migration in which interneuron subtypes migrate to specific brain regions (likely driven by guidance factors) where they will reside and avoid other brain regions which do not support their maturation. Alternatively, interneuron subtypes could be diffusely dispersed throughout multiple brain regions followed by selective survival (or selective death) of subtypes via apoptosis during the first 2 postnatal weeks¹² (Figure 1). The challenge was to develop an approach to assess these mechanisms.

Our strategy to determine how brain environments regulate interneuron fate and clearly differentiate this from intrinsic genetic programs required two steps. First, we identified significant differences in either the proportion or the presence/absence of specific interneuron subtypes between distinct brain regions (in our case, the cortex and hippocampus). Second, we devised a strategy to harvest immature interneuron precursors from the cortices or hippocampi of early postnatal brains and challenge them in new brain environments. Many previous studies have successfully grafted MGE cells into a variety of brain regions¹³ but this approach is inadequate for exploring environmental changes because it loses the relationship between interneuron subtype and brain region. By harvesting interneuron precursors at P0–P2, we circumvent this problem by obtaining cells that have migrated to their final destination but their exposure to local environmental cues is limited. Upon transplantation into new environments, we could determine whether transplanted cells

adopted features of their new “host” environment or maintained features representative of their original “donor” brain region.

We used *Nkx2.1-Cre;Ai9* mice to select MGE-derived interneurons, which consist of the largely nonoverlapping SST⁺, PV⁺, and neuronal nitric oxide synthase-expressing (nNOS⁺) populations. The endogenous cortex contains a very small percentage of nNOS⁺ interneurons (<5%), whereas the hippocampus contains an equivalent proportion of SST⁺, PV⁺, and nNOS⁺ cells. We classified grafted tomato⁺ cells based on electrophysiological and neurochemical markers 30 days after transplantation. Our results indicate that the proportion of these interneuron subgroups populating a specific brain region is primarily determined by the host environment.¹ For example, cortex-to-hippocampus grafts produced a SST⁺/PV⁺/nNOS⁺ ratio that was nearly identical to the endogenous hippocampus or hippocampus-to-hippocampus grafts, with the opposite result for hippocampus-to-cortex transplants. These results are consistent with (1) the cardinal-definitive specification hypothesis, whereby the environment is inducing the differentiation of specific subtypes and/or (2) the selective survival/death hypothesis in which the host environment selects for (or against) previously fated subtypes (Figure 1).

We then explored the distribution of more specific interneuron subtypes. There are two types of nNOS⁺ interneurons that can be classified neurochemically: type I nNOS⁺ cells express high levels of nNOS⁺ and coexpress SST⁺, whereas type II nNOS⁺ cells display weak nNOS levels and represent the

neurogliaform and ivy interneuron populations.¹⁴ The small proportion of endogenous cortical nNOS⁺ MGE-derived cells consist of both type I and type II nNOS⁺ interneurons, whereas the hippocampus contains only type II nNOS⁺ cells. Our grafting experiments revealed that a mix of type I and type II nNOS⁺ cells was observed in the cortex-to-hippocampus grafts, whereas only type II nNOS⁺ cells were present in hippocampus-to-hippocampus transplants.¹ Thus, the proportion of type I and type II nNOS⁺ grafted cells strongly reflects the donor region, with cortically residing type I nNOS⁺ cells surviving and maturing in the hippocampus. In contrast to the previous results, these findings argue against both the cardinal-definitive specification and the selective survival/death hypothesis because type I nNOS⁺ cells should not survive in the hippocampus in these scenarios.

However, selective migration of nNOS⁺ interneuron precursors is consistent with these results. In this model, type I nNOS⁺ cells initially migrate into the cortex and do not enter the hippocampus (Figure 1). To investigate this hypothesis, we immunostained P1 brains for nNOS and found that type I nNOS⁺ cells were restricted to the cortex and not found in the hippocampus (whereas we did not detect and type II nNOS⁺ cells in either brain region at this age).¹ Thus, cortex-to-hippocampus grafts already contain type I nNOS⁺ cells at P1, whereas hippocampus-to-hippocampus grafts do not. The other implication from these results is that hippocampus does not actively select against type I nNOS⁺ cells; otherwise, they should not survive the transplantation. Instead, the hippocampus never contains type I nNOS⁺ cells under normal conditions and does not reject this transplanted subtype.

Our results indicate that type I nNOS⁺ MGE-derived interneurons are fated during embryogenesis and undergo targeted migration specifically to the cortex. Does this mechanism hold true for all other interneuron subtypes? There is some evidence for early specification and targeted migration of chandelier cells,^{10,15} and MGE-derived striatal interneurons display a genetic cascade that directs early fate decision and prevents migration to the cortex.^{16–19} However, the disconnect between the mature gene expression and the developmental transcriptome complicates this issue. Although recent studies have classified the transcriptome of mature interneuron subtypes,²⁰ many mature interneuron markers used in the field (PV, calretinin, vasoactive intestinal peptide, etc) are not expressed during embryogenesis or in neonates. Thus, until early subtype-specific markers are identified,⁹ it is very difficult to assess the extent to which every interneuron subtype is fated during embryogenesis.

These findings present an interesting contrast whereby global interneuron proportions are determined by the host environment while the presence of early fated region-specific subtypes correlates with the donor region. Not surprisingly, the three models we proposed are overly simplistic, and different aspects of interneuron fate determination and maturation likely require a combination of these mechanisms. Similar to our results, a recent study found that chandelier cells adopted host-specific

connectivity patterns when heterotopically transplanted between the cortex and the hippocampus despite a clear difference in birthdate and developmental trajectory.¹⁵ Both this study and our findings highlight how the environment can sculpt interneuron maturation of early fated interneurons, but many questions remain. Are these findings applicable to other brain regions, as there are more significant differences in interneuron subtypes and connectivity in the striatum compared with the cortex and hippocampus? Would the environmental influence change in heterochronic transplants (eg, harvesting P2 interneurons and transplanting into P7 pups)? Is there a critical period during maturation whereby the environmental influence over heterotopic transplants would be minimized? Do our general conclusions regarding interneuron fate and maturation apply to the development of other neuron classes? We hope that other labs will use and improve on these strategies to explore the challenging question of how intrinsic genetic programs collaborate with environmental cues to direct neuronal fate and maturation.

Acknowledgements

The author thanks Dr Giulia Quattrococo for comments on this manuscript.

Author Contributions

TJP wrote and developed the arguments presented in this Commentary.

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