CellPress

Plug and Play Brain: Understanding Integration of Transplanted Neurons for Brain Repair

Shane Grealish[1] and Malin Parmar[1,*]
[1]Developmental and Regenerative Neurobiology, Wallenberg Neuroscience Center, and Lund Stem Cell Centre, Department of Experimental Medical Science, Lund University, 22184 Lund, Sweden
*Correspondence: malin.parmar@med.lu.se
http://dx.doi.org/10.1016/j.stem.2016.11.008

In a recent issue of *Nature,* Falkner et al. (2016) use chronic two-photon imaging, virus-based transsynaptic tracing, and dynamic calcium indicators to elegantly demonstrate extensive in vivo functional maturation and target-specific functional integration of transplanted embryonic mouse cortical progenitors into adult lesioned visual cortical circuits.

Very few new neurons are formed in the adult human brain (Ernst and Frisén, 2015), and this lack of endogenous repair means that the adult brain has a very limited capacity to replace neurons lost to disease or injury, leading to life-long disease and disability. In recent decades, brain repair strategies based on cell replacement therapy have made significant progress for a number of neurological disorders. Parkinson's disease and Huntington's disease have been prime targets due to the focal degeneration of dopamine and medium-spiny GABAergic neurons, respectively. The first animal experiments using transplanted rodent fetal brain tissue were performed already in the 1970s (Björklund and Stenevi, 1979). The extension of this approach to using human fetal tissue opened up the possibility for clinical translation, which ultimately led to a number of clinical trials in patients with Parkinson's and Huntington's disease (reviewed in Barker et al., 2013; Rosser and Bachoud-Lévi, 2012). Due to the complexity of the organization of the cerebral cortex in terms of highly specific topography and connectivity, replacement of lost neurons is a more challenging task, and is thus still at the pre-clinical stage.

One caveat in the field of cell-based brain repair is that we have limited insight and insufficient technologies to achieve understanding of how well the transplanted neurons mature and functionally integrate in the host brain, an environment not posed to nurture new neurons or provide guidance cues for correct innervation. Recent work using donor tissue from transgenic reporter mice has revealed that in fact the adult brain is a growth-permissive environment that allows for highly specific and target-directed axonal outgrowth (Thompson and Björklund, 2015). Previous studies using fetal cortical progenitors transplanted into lesioned adult mice showed that circuit reconstruction of the motor pathway is possible, both histologically and functionally (Fricker-Gates et al., 2002; Gaillard et al., 2007). Moreover, the grafted cortical progenitors retained their capacity for target-specific outgrowth regardless of which cortical area they were transplanted to—e.g., visual cortical progenitors innervating visual target structures when placed in the motor cortex—which holds promise for future brain repair strategies, including more complex diseases such as stroke.

By expanding on these findings further using a highly selective photolytic lesion technique in combination with advanced methods for in vivo assessment of neurons, Falkner et al. were able to make a number of important observations of how new neurons mature, integrate, and function after transplantation into the damaged visual cortex (summarized in Figure 1).

First, the data support previous findings that the transplanted neuroblasts survived well and adapted a similar regional identity and morphology to that of the endogenous L2/3 V1 neurons. In vivo two-photon imaging was used to show how the new neurons matured phenotypically and structurally over time and also confirm that the transplanted neurons were able to innervate appropriate target structures, such as the retrosplenial and entorhinal cortices and the contralateral primary visual cortex. Importantly, the temporal dynamics of synapse formation and pruning was observed longitudinally in this study, revealing that this active process continues with appropriate apical and basal dendrite maturation over several weeks after transplantation before reaching a steady state following the time course observed during normal development.

Second, synaptic circuit integration was confirmed using *trans*-synaptic tracing technology. A modified rabies virus was used to retrogradely label the host neurons that form synaptic contacts with the transplanted neurons, revealing that the endogenous host neurons of the surrounding visual cortex and dorsal lateral geniculate nucleus formed synapses with the newly integrated transplanted neurons. Furthermore, the innervation and synaptic contacts with the transplanted neurons were topographically appropriate—which is a key feature of the visual circuit—confirming that the adult host visual cortex has the ability to integrate and accept the newly transplanted cells in a circuit-specific manner.

Third, in order to probe if the synaptic integration of the transplanted neurons was functional, the authors used different visual stimuli to assess response encoding at the single-cell level using genetically encoded calcium indicators. Individual transplanted neurons were shown to exhibit strong preferential responses to visual stimuli of different orientation and direction that further refined over several weeks, proving that the transplanted neurons had become functionally integrated within the visual circuit and acquired an appropriate functional role that was plastic and matured over time, eventually

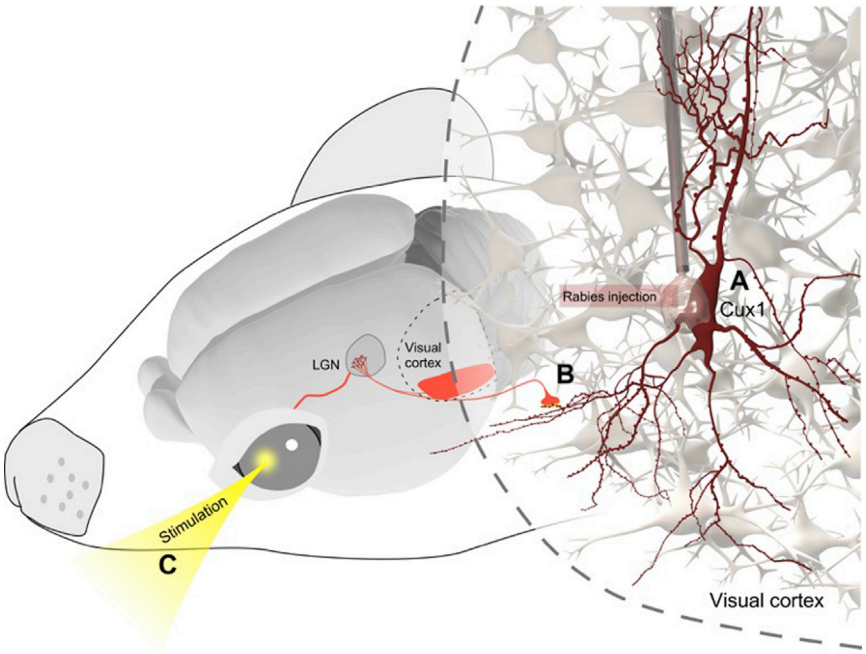

Figure 1. Schematic Overview Summarizing the Main Findings of the Study
(A) Transplanted neuroblasts adapted a similar regional identity and morphology to that of the endogenous L2/3 V1 neurons as exemplified by their morphology and expression of Cux1.
(B) Synaptic circuit integration was performed using modified rabies virus, which confirmed connections from surrounding visual cortex and dorsal lateral geniculate nucleus.
(C) Transplanted neurons responded to distinct visual stimuli of the ipsilateral or contralateral eye, indicative of functional integration within the existing host visual circuit.

becoming virtually indistinguishable from endogenous primary visual cortical L2/3 neurons.

In summary, this elegantly performed study has revealed how newly transplanted neurons functionally mature and integrate into already established, yet damaged, functional neuronal circuits in real-time in vivo. Moreover, the level and specificity of integration was unprecedented, truly confirming that transplanted cells can be used to repair a damaged component of circuitry within the adult brain—rewiring appropriately and repairing the overall circuit with correct inputs and outputs.

In this era, when stem cells are at the verge of clinical translation, cell-based brain repair is gaining new momentum. As the field rapidly moves toward developing new stem cell-based therapies, the ability to understand how new neurons functionally integrate and mature in the adult brain is pivotal for successful outcomes in such stem cell-based therapies. The Falkner et al. study was conducted using mouse fetal neurons transplanted into a lesioned mouse circuitry with a level of sophistication that has never been achieved before. Promising work is already underway to generate cortical progenitors from pluripotent stem cells with the capacity to integrate and connect with the host brain after transplantation to the lesioned cortex (Espuny-Camacho et al., 2013; Michelsen et al., 2015). The demonstrated ability of the new neurons to integrate, connect, and take over the function of the lost neurons holds great promise for future stem cell-based therapies. Now, more work is needed to define the window of opportunity for repair, better understand the underlying mechanisms, and identify the critical factors in the transplanted cells and in the damaged brain that control the functional integration and maturation that lead to repaired neural circuitry and, ultimately perhaps, to new reparative therapies for otherwise incurable diseases.

ACKNOWLEDGMENTS

The authors would like to thank Bengt Mattsson for scientific illustration and Prof. Anders Björklund and Dr. Eleanor Grant for input and discussion. M.P. is funded by the European Research Council ERC Grant Agreement n. 309712.

REFERENCES

Barker, R.A., Barrett, J., Mason, S.L., and Björklund, A. (2013). Lancet Neurol. *12*, 84–91.

Björklund, A., and Stenevi, U. (1979). Brain Res. *177*, 555–560.

Ernst, A., and Frisén, J. (2015). PLoS Biol. *13*, e1002045.

Espuny-Camacho, I., Michelsen, K.A., Gall, D., Linaro, D., Hasche, A., Bonnefont, J., Bali, C., Orduz, D., Bilheu, A., Herpoel, A., et al. (2013). Neuron *77*, 440–456.

Falkner, S., Grade, S., Dimou, L., Conzelmann, K.K., Bonhoeffer, T., Götz, M., and Hübener, M. (2016). Nature *539*, 248–253.

Fricker-Gates, R.A., Shin, J.J., Tai, C.C., Catapano, L.A., and Macklis, J.D. (2002). J. Neurosci. *22*, 4045–4056.

Gaillard, A., Prestoz, L., Dumartin, B., Cantereau, A., Morel, F., Roger, M., and Jaber, M. (2007). Nat. Neurosci. *10*, 1294–1299.

Michelsen, K.A., Acosta-Verdugo, S., Benoit-Marand, M., Espuny-Camacho, I., Gaspard, N., Saha, B., Gaillard, A., and Vanderhaeghen, P. (2015). Neuron *85*, 982–997.

Rosser, A.E., and Bachoud-Lévi, A.C. (2012). Prog. Brain Res. *200*, 345–371.

Thompson, L.H., and Björklund, A. (2015). Neurobiol. Dis. *79*, 28–40.