Journal Club

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Not Every Graft Has What It Takes to Attract a Mossy Fiber

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Review of Shetty et al. (<http://www.jneurosci.org/cgi/content/full/25/37/8391>)

Although the adult mammalian brain maintains high levels of structural plasticity and can change dramatically in response to neural activity (Zito and Svoboda, 2002), not all structural change is adaptive. For example, neural activity in status epilepticus is associated with several structural abnormalities. One consequence of limbic seizures in animal models and in human temporal lobe epilepsy (TLE) is the excitotoxic death of neurons in the CA3 region and in the hilus of the dentate gyrus and the ensuing aberrant mossy fiber sprouting (when axon collaterals from dentate gyrus granule cells invade the molecular layer and establish abnormal connections with other neurons, including excitatory kainate-receptor-driven synapses on granule cells themselves) (Scharfman et al., 2003; Epszstein et al., 2005). Acute seizures also enhance the level of neurogenesis in the adult dentate gyrus, but some of the newly born cells form abnormal connections after migrating to ectopic locations in the hippocampus (Parent and Lowenstein, 2002). Both mossy fiber sprouting and epilepsy-induced neurogenesis may facilitate future seizure activity. Thus, an effective treatment for human TLE might have to address not only the problem of replacing degenerated cells, but also the issue of repairing the abnormal connectivity associated with this condition. Indeed, in a re-

cent study in *The Journal of Neuroscience* (Shetty et al., 2005), the authors succeeded in inhibiting aberrant mossy fiber sprouting with fetal brain cell transplants in a rat model of TLE. Shetty et al. established the necessity of homotypic neurons in the grafts (CA3, but not CA1, pyramidal cells) and gathered important information regarding the temporal window during which the transplant can be introduced (4–45 d after damage to the hippocampus). Finally, the authors provide evidence for the effectiveness of a CA3 xenograft from the mouse to the rat and use the xenograft to map out differences in connectivity between transplants containing CA3 versus CA1 cells.

The authors began with a unilateral intracerebroventricular injection of kainic acid (KA) into the brains of adult rats (Fig. 1). This excitotoxic agonist for the AMPA/KA glutamatergic receptor leads to limbic seizures and cell death in the CA3 region and in the hilus. Applying Timm's silver sulfide stain, which labels zinc-containing vesicles in axonal fibers, to sections of the hippocampus, the authors confirmed that mossy fibers invaded the dentate gyrus supragranular layer in lesioned animals, but not in controls [Shetty et al. (2005), their Figs. 1A,B (<http://www.jneurosci.org/cgi/content/full/25/37/8391/FIG1>) and 2A,B (<http://www.jneurosci.org/cgi/content/full/25/37/8391/FIG2>)].

Meanwhile, Shetty et al. harvested brains of rats on embryonic day 19 and prepared cell suspensions containing either CA1 or CA3 neurons. Then, at two different time points after KA injection, 4

or 45 d, the authors introduced the transplants, placing the grafts as close as possible to the degenerated CA3 layer. Shetty et al. waited an entire year before looking at hippocampal structure of the rats with the transplants. Because 1 year comprises a substantial part of the rat's life span (typically <3 years), this timing allowed the authors to gather information about the relatively long-term outcome after transplants. Nuclear Nissl staining confirmed lesion effectiveness in the lesion-only group and was used to localize transplants in the grafted animals. New transplanted neurons were evident in animals that received grafts containing either CA1 or CA3 pyramidal cells. Interestingly, in a separate experiment, the authors found that the CA1 and the CA3 cells within the transplants exhibited divergent and cell-type-specific patterns of axonal growth. Transplanted CA3 cells projected heavily to the CA1 field and other regions typically innervated by CA3 pyramidal neurons. On the other hand, transplanted CA1 cells, while still projecting to some of the same regions, showed connectivity patterns that were more similar to those of normal CA1 pyramidal cells [Shetty et al. (2005), their Figs. 4 (<http://www.jneurosci.org/cgi/content/full/25/37/8391/FIG4>), 5 (<http://www.jneurosci.org/cgi/content/full/25/37/8391/FIG5>), and 6 (<http://www.jneurosci.org/cgi/content/full/25/37/8391/FIG6>)]. Shetty et al. traced axonal projections from their grafts by using a cross-species transplantation procedure, which involved grafting mouse CA1 or CA3 cells into the hippocampus of adult KA-lesioned rats. This

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permitted the authors to stain neural tissue for the mouse-specific protein M6 that is present in neuronal cell bodies but is highly concentrated in the axons. Beyond providing a convenient method for tracing the circuitry established by the transplanted neurons, this experiment lends some support to the possibility of clinical use of xenografts, which is particularly valuable in light of the regulatory considerations associated with the use of human fetal brain tissue.

Although the goal of neuronal replacement per se was accomplished with the transplants containing either CA1 or CA3 neurons, the degree of mossy fiber sprouting, measured either as the width of the mossy fiber bundle or as the density of Timm's granules, differed dramatically between the two types of grafts. With the CA1 grafts, administered either 4 or 45 d after KA injection, the amount of mossy fiber sprouting 1 year later was the same as in animals that received lesions alone. Thus, while the transplanted cells had integrated into the new circuitry, epileptogenic architecture persisted in the dentate gyrus. However, the CA3 grafts effectively decreased mossy fiber sprouting when administered early (4 d) or relatively late (45 d) after hippocampal injury [Shetty et al. (2005), their Figs. 1C–E, 2C,D, and 3 (<http://www.jneurosci.org/cgi/content/full/25/37/8391/FIG3>)]. The authors posit a likely involvement of chemoattractant and chemorepellant molecules as the basis for the differential capacity of the two types of transplants to rewire the mossy fiber pathway. If that is so, then the age of the graft donor becomes an important issue: neither very early embryonic nor adult stem cells may be as useful for treating aberrant mossy fiber sprouting as fetal grafts containing CA3 neurons, at least until we acquire a much deeper understanding of the chemical factors involved in axonal guidance.

In summary, Shetty et al. elucidated the following novel function for fetal brain cells that goes beyond cell replacement: to reconfigure potentially well established aberrant circuits by providing appropriate postsynaptic targets. These

Figure 1. Simplified schematic of the experiments and the results presented by Shetty et al. (2005). *A*, In an intact hippocampus, the axons of dentate gyrus cells, or mossy fibers, project heavily to CA3 pyramidal cells. *B*, After an injection of KA, mossy fibers sprout into supragranular layer, where some axonal collaterals form potentially epileptogenic excitatory connections with granule cells. Aberrant mossy fiber sprouting recedes after transplants containing CA3 (*C*), but not CA1 (*D*), neurons.

experiments make an important scientific contribution to the search for a cure for TLE and other types of seizures, yet many questions remain for future investigation. For one, how wide is the temporal window during which the graft must be administered to inhibit mossy fiber sprouting? More importantly, does the degree of mossy fiber sprouting inhibition in KA-lesioned animals after transplants actually correlate with functional or behavioral recovery? This question can be pursued explicitly in animals that, unlike the ones used in this series of studies, show a substantial level of spontaneous recurrent seizures. In addition to assessing seizure frequency in animals before and after the transplant, it would be interesting to examine aspects of hippocampal-dependent learning in these animals. Although the likely clinical applications of the experiments by Shetty et al. remain to be seen in the future, we have already learned from their work the following key lesson in basic neuroscience: the strength of a neuron's identity, even in the case of a very

young cell, can override the chemical drive of the surrounding milieu.

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