Assembling Cell Ensembles

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The way the hippocampus processes information and encodes memories in the form of "cell assemblies" is likely determined in part by how its circuits are wired up during development. In this issue, Xu et al. now provide new insight into how neurons arising from a single common precursor migrate to their final destination and form functionally synchronous ensembles.

The hippocampus has intrigued neuroscientists for decades because of its known role in learning and memory as well as spatial navigation (Buzsáki and Moser, 2013). Experimental studies in mice have documented that hippocampal neurons process and encode spatial locations in the form of populations of neurons that spike when the animal is in a certain place, resulting in spike sequences corresponding to the animal's movement along a trajectory. Sixty-five years ago, Donald Hebb proposed that groups of synchronously active neurons encoding related information, or "cell assemblies," could form the neural basis of memory through strengthening of their interconnections (Hebb, 1949). Although it is commonly presumed that these cell assemblies are formed by experience-dependent synaptic plasticity, a number of recent findings suggest that circuit motifs established during development may also influence the formation of cell assemblies. In this issue of *Cell*, Xu and colleagues (2014) analyze the formation of hippocampal circuits from dividing cells, providing new insight into the formation and function of cell ensembles that may contribute to the formation of cell assemblies in the hippocampus.

Little is known about how cells and connections are arranged and assembled during hippocampal (archicortex) development. More is known about the evolutionarily more recent neocortex, which has a columnar structure consisting of multiple layers of cell bodies (Nadarajah and Parnavelas, 2002). These layers are formed in an inside-out fashion, with the deepest layers formed first, and subsequent layers formed by cells migrating through the deeper layers to form more superficial layers. Cell migration occurs by two mechanisms: vertical migration along the fibers of radial glial cells (RGCs) and horizontal migration after neurons separate from the RGC. The columnar structure of neocortex is established during development as a result of the strict vertical orientation of RGC fibers and the short distances of horizontal migration. Clonally related neurons arising from the terminal divisions of a common precursor for the most part remain within a single column.

The evolutionarily older hippocampus is in some ways simpler than the neocortex. It consists of a single, tightly packed layer of cell bodies, just a few cell bodies deep. As in the neocortex, the deepest cells are born first, and superficial cells must migrate through the deeper layers to reach their final destination (Frotscher and Seress, 2007). Although the hippocampus lacks a clear columnar organization, if RGC fibers are vertically oriented and horizontal migration is limited, as in the neocortex, there could be a hidden columnar organization defined by the positioning of sibling neurons (Figure 1A). However, Xu and colleagues show that this is not the case. They identify a novel mechanism by which dividing neurons deviate from columnar organization in the hippocampus. Furthermore, they go on to show that these horizontally distributed sibling neurons have an interesting functional relationship.

To study hippocampal development, the authors took advantage of molecular tricks that allowed them to express a fluorescent protein in cells arising from a common precursor. By inducing expression at specific times, they were able to label dividing cells at different stages and, by weak induction, they were able to label small numbers of separate clones; that is, clusters of cells presumably arising from an individual labeled precursor were visible against a background of mostly unlabeled cells. An important step in their experimental design was to do some of the labeling at embryonic day 12–13 (E12–13). At this stage (one week before birth), many neuroepithelial cells (neural stem cells) have produced RGCs that divide asymmetrically to spawn intermediate progenitors (IPs) or postmitotic neurons. IPs can also divide (likely just once) to produce two postmitotic neurons. Sparse labeling at E12–13 thus results in isolated clusters of fluorescent cells (i.e., a clonal family). Soon after labeling (e.g., E14) a family consists of an RGC and a couple of IPs and/or immature neurons. Also at this stage, each RGC extends a fiber vertically, along which the IPs and neurons migrate. About two days later (E16), labeled immature astrocytes are observed, which are presumably spawned by later division of the RGC. Each family can be observed at later embryonic stages (e.g., E18) or even postnatally, and typically consists of one RGC, four to five neurons, and in some cases an astrocyte (Figures 1B and 1C).

Using this strategy, Xu and colleagues made two key discoveries. First, they found that the RGC fibers began to bend significantly at E16 (Figure 1B). This bending became more pronounced in the last few days of embryonic development (Figure 1C) and nearly horizontally oriented segments were observed even after birth. It is unclear whether this bending is caused by active movement of RGC cell bodies or end feet, or whether

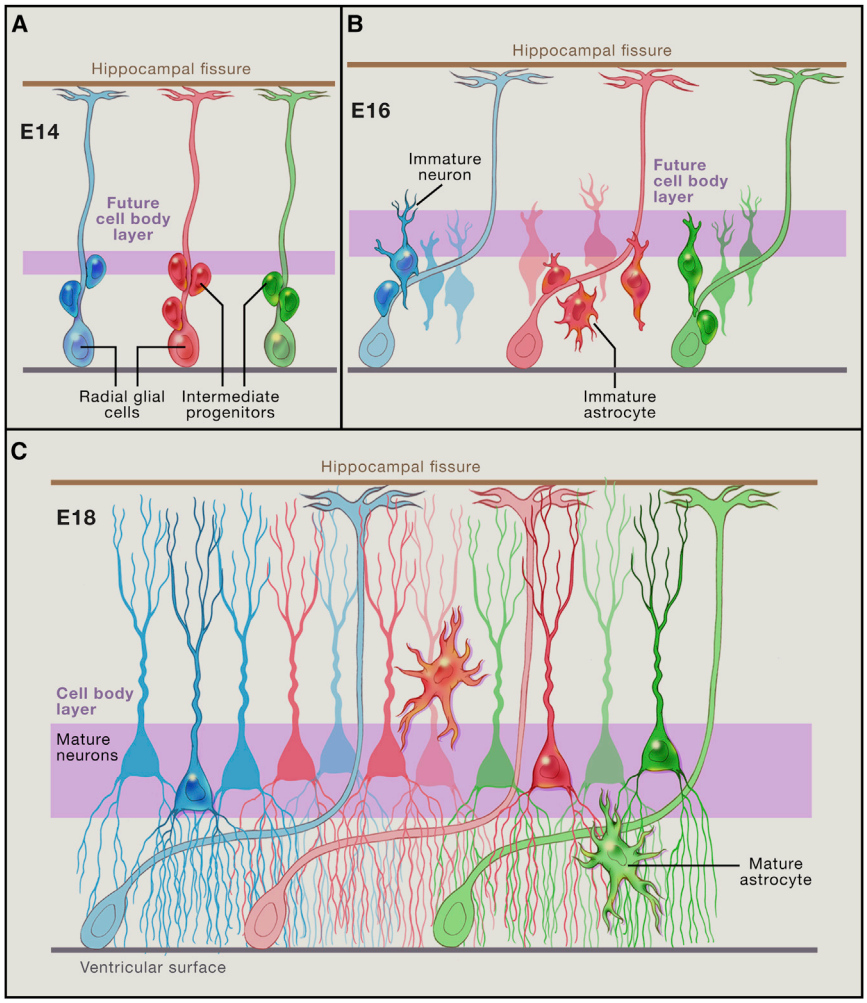

Figure 1. Schematic Depiction of Neuronal Development in the Hippocampus

(A–C) Neuroepithelial cells near in the ventricular zone give rise to radial glial cells (RGCs) with a long process extending up to the hippocampal fissure. RGCs in turn divide to produce postmitotic neurons or intermediate progenitors (IPs) that later become neurons. Each color depicts a clonal cell line originating from an RGC labeled at embryonic day 12 (E12). (A) At E13-14, RGCs are oriented vertically. (B) By E16, RGC processes begin to bend, IPs begin to differentiate into neurons, and RGCs divide further, giving rise to astrocytes. (C) At E18 and later, RGC bending is more pronounced and neurons and astrocytes begin to take a more mature form and separate from the RGC.

both ends are fixed and the fiber bends as new cells are born and the tissue expands. Importantly, the authors also observed IPs and immature neurons that appeared to be migrating along the angled portion of the RGC fiber. As this bending progressed, morphologically more mature neurons in the family were observed to have separated from the RGC fiber, even while others continued to be associated with it (presumably migrating along it). As a result, each clonally related family of neurons was found to be horizontally distributed in the eventual cell-body layer, with 90% of the neurons located 200 μm or less from their nearest sibling (Figure 1C). Thus, unlike in the neocortex, RGC fibers in the hippocampus bend significantly, which contributes to the horizontal distribution of clonally related neurons. However, embryonic neurons have been shown by others to move vertically by interacting with the fibers of multiple RGCs (Kitazawa et al., 2014). Thus, it is possible that neurons can migrate vertically, away from the parent RGC fiber, and that movement of the RGC cell body contributes to both the bend of its fiber and the horizontal spread of its daughter cells.

The second key discovery was that clonally related neurons have a high probability of receiving synchronous inhibition, as assayed using quadruple patch-clamp recordings in hippocampal slices. This is important, not only because it suggests that clonally related neurons may exhibit synchronous activity in vivo, but also because it demonstrates that clonally related neurons are recognizable by the axons of other neurons forming synapses onto this population. It is not known whether synchronous excitatory input is also established by these mechanisms. Although the authors did not observe it in slices, the possibility remains that sibling neurons receive common inputs from excitatory neurons that are not spontaneously active in slices. Yet another interesting aspect of their findings is that the functional relationship of clonal siblings in the hippocampus is very different from what the same lab has observed in neocortex, where clonally related neurons are preferentially interconnected by transient electrical synapses followed by stable chemical synapses (Yu et al., 2009, 2012).

The authors' findings about development of the hippocampus also have the potential to contribute to a deeper understanding of its function. The possibility that clonally related sibling neurons may exhibit synchronous activity in vivo raises the possibility that they may also have common response properties (as in Li et al., 2012), such as firing when the animal is at similar locations in the environment. If so, clonal siblings could serve as a mechanism for representing information redundantly in the hippocampus. Furthermore, several studies involving recordings in freely moving animals suggest that the "prewiring" of hippocampal circuits during development may contribute to the response properties of hippocampal neurons, which seem to have firing properties that reflect internal activity as much as sensory input (Buzsáki and Moser, 2013; Dragoi and Tonegawa, 2011). The current findings suggest that the blueprint for ensembles of synchronously active neurons could be laid down during embryonic development.

Finally, the new results raise a number of questions about the mechanisms by which clonally related ensembles are formed. What molecular cues and physical forces are responsible for the various movements involved, such as migration of the IPs, neurons, and astrocytes along the RGCs, bending of the RGCs, and separation of the RGCs and the neurons as the latter mature into full-blown pyramidal neurons? Not only will the answers to these questions illuminate the molecular mechanisms by which neuronal ensembles (and possibly cell assemblies) are formed, but they may provide clues to the origins of diseases in which miswiring is known to play a central role, including autism and schizophrenia.

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The Angiotensin II Type 2 Receptor for Pain Control

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All well-known deleterious effects of angiotensin (Ang) II, including vasoconstriction, inflammation, water and salt retention, and vascular remodeling, are mediated via its type 1 (AT_1) receptor. This explains why AT_1 receptor blockers (ARBs) and inhibitors of Ang II synthesis, such as ACE inhibitors and renin inhibitors, are beneficial for cardiovascular disease. Yet, Ang II has a second receptor, the Ang II type 2 (AT_2) receptor, the function of which, even after over 20 years of research, remains largely unknown. In this issue, Marion et al. provide a new chapter to the AT_2 receptor story.

Previously, it was proposed that Ang II type 2 (AT_2) receptors antagonize the effect of the AT_1 receptor, and that the beneficial effects of ARBs could be due to the stimulation of the unoccupied AT_2 receptor. However, subsequent studies revealed that AT_2 receptors are not always protective (Verdonk et al., 2012a), and the AT_2 receptor agonist C21, at AT_2 receptor-selective doses, does not lower blood pressure (Verdonk et al., 2012b). Of interest, AT_2 receptors were shown to stimulate neurite outgrowth, and AT_2 inhibitors to reduce pain signaling in animal models and in rodent and human sensory neurons in vitro (Anand et al., 2013) (Figure 1A). As such, they are potential targets for agonists in nerve regeneration and for antagonists to suppress pain. Recently, the latter concept has been successfully tested in patients with postherpetic neuralgia, where the AT_2 receptor antagonist EMA401 in a randomized, double-blind, placebo-controlled phase 2 trial significantly reduces pain over 4 weeks of treatment (Rice et al., 2014). This is the only human trial so far involving drugs acting on AT_2 receptors, which suggests that AT_2 receptor antagonists might be further developed to treat neuropathic pain.

In this issue of *Cell*, Marion et al. (2014) now suggest that AT_2 receptor stimulation may induce analgesia. Following the clinical observation that *Mycobacterium ulcerans*, the etiological agent of Buruli ulcer, causes extensive skin lesions that are not accompanied by pain, they study a mouse footpad model in which pain