Commentary

The increasingly plastic, hormone-responsive adult brain

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Pity the poor neuroscientist. Drilled in details of neuroanatomy that have been gleaned from over a century's intensive research, we come to believe we have a reasonably accurate portrait of what the cells constituting the brain look like, and roughly what their connections are with one another. By 1949, Donald Hebb (1) persuasively described how the behavioral plasticity we see in adult animals, such as that exemplified in learning and memory, could be accomplished, in theory, by simply changing the strength of existing synapses, without any rewiring of the circuits. But it has become clear that, in fact, synaptic connections come and go even in the adult brain, and that our snapshot views of the brain at a single point in time may be missing the point. Indeed, reports from the annual assembly of some 25,000 congregants at the Society for Neurosciences meeting suggest that the adult brain is each year more plastic than it was the year before. In several systems, hormones play a role, fine tuning nervous system connections to facilitate, at the appropriate time, adaptive behaviors such as singing (2), copulation (3), pheromone detection (4), stress response (5), etc. In this issue of PNAS, Yankova and colleagues (6) report that the hormonally driven appearance of new synapses in one brain region, already shown to wax and wane in the course of just 4 days in the estrous cycle of female rats, is not a mere duplication of connections between two established partners. Rather, it appears that the new synapses represent a divergence of connections that may serve to synchronize neural activity, a circuit change that might prove relevant to epilepsy and/or the cognitive benefits of estrogen treatment.

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This story began over a decade ago with the fortuitous aggregation of graduate student Catherine Woolley and postdoctoral fellow Elizabeth Gould in Bruce McEwen's laboratory at The Rockefeller University. Using a Golgi technique refined by Gould (7), they examined the CA1 region of the hippocampus, counting the number of spines on dendrites of pyramidal neurons. These dendritic spines are an important point of excitatory synaptic input to these hippocampal neurons. Woolley examined adult female rats and found that the number of dendritic spines displayed by pyramidal neurons of the CA1 region of the hippocampus varied with the estrous cycle (8). This finding was a surprise for several reasons. The rat estrous cycle is only 4–5 days long, so this synaptic turnover was happening, cyclically, in a matter of days. Furthermore, when they looked at the right point in the cycle, about 30% of the spines disappeared in just 1 day. Because the spines are an important site for synaptic connections, their result suggested that synapses were coming and going in the course of hours, an idea confirmed with electron microscopy showing that the new spines were indeed hosting synapses. It was also a bit surprising that the hippocampus, a structure associated with memory consolidation and spatial reasoning, should exhibit plasticity across the estrous cycle. An earlier report (9) that synapses were appearing and disappearing across the cycle in the rat arcuate nucleus of the hypothalamus was newsworthy for the rapidity of the change, but that brain region was known to be involved in regulation of the estrous cycle. That such rapid changes were also taking place outside the traditional hormone-sensitive brain regions was unexpected.

Subsequently, Gould would further challenge our ideas about the limits of neural plasticity, reporting that new neurons may arise in adulthood in brain regions such as the cerebral cortex (10), where the number of neurons was traditionally thought to be fixed early in life. Meanwhile, Woolley pursued the waxing and waning hippocampal synapses.

Woolley (11) next asked, "What is fluctuating during the estrous cycle to drive these synaptic changes?" Because the spines were more numerous during those parts of the cycle when estrogen secretion is high, she removed the ovaries from adult females and treated them with either estrogen or nothing. The estrogen-treated females displayed increased spine density in the CA1, compared with untreated animals. So it is the cyclical appearance of estrogen in the course of the estrous cycle that drives dendritic spine formation.

We don't know why the female rat's hippocampus undergoes this cyclic remodeling, but presumably it facilitates reproduction in some manner. Because the hippocampus contributes to spatial learning and memory consolidation, perhaps the changes hone navigational skills and/or spatial recall when the female rat must find the male(s) she has chosen for mating.

When these new spines arise on the dendrites of estrogen-treated females, which cells are synapsing on them? Do the new spines receive input from preexisting presynaptic terminals, or do new terminals arise to communicate with them? Woolley *et al.* (12) began exhaustive study of serial sections with electron microscopy and found that estrogen treatment not only increased the density of spines, but also increased the frequency of presynaptic terminals that seemed to synapse with several spines rather than just one spine. In fact, the numbers suggested that perhaps all of the new spines were receiving input from a preexisting axon terminal that was now synapsing on two (or more) spines, including the new one. In the authors' terms, estrogen treatment converted some presynaptic boutons that had innervated only a single spine (single synapse boutons, or SSBs) into boutons innervating multiple spines (multiple syn-

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apse boutons, or MSBs), and had increased the average number of synapses made by MSBs. Thus, it appears that existing presynaptic terminals, which were monogamously innervating a single dendritic spine when estrogen levels were low, become bigamous when estrogen levels rise, innervating both their old partner and a newly formed spine. The most parsimonious explanation would be that the new connections simply increased the impact of preexisting connections between a particular afferent and a particular CA1 dendrite.

This result suggests that no new *pattern* of innervation is formed, simply a multiplication of existing connections. But this new paper shows that things are not that simple. Yankova *et al.* address this question by filling individual CA1 pyramidal cells with biocytin to label the set of spines from a particular postsynaptic neuron. They then use electron microscopy to reconstruct a stretch of dendrite, finding all of the labeled spines and the terminal boutons that innervate them. Again, they find that estrogen treatment increases the number of spines and also increases the number of presynaptic boutons innervating multiple spines. But now they can ask about the pattern of innervation that results. When these preexisting boutons synapse on new spines, are they innervating spines from the *same* CA1 cell, or are they innervating spines from two different cells? Operationally, the question is whether the two spines innervated by a single bouton are both filled with the biocytin marker, or is only one filled? Yankova *et al.* found that the vast majority of presynaptic boutons innervating multiple spines were innervating spines from *different* postsynaptic cells. Because these multiple synaptic boutons seem to account for all of the new spines, this result shows that the new synapses in fact serve to change the pattern of innervation. Boutons that previously innervated only one spine are now preferentially forming an additional synapse with a new spine from a different CA1 cell. The authors cannot say whether the two cells communicating across the new synapse had no prior connections to each other anywhere, because that would require the reconstruction of the entire CA1 pyramidal cell and all of the neurons innervating it. But they can say that the pattern of innervation is not a simple multiplication of inputs: a bouton that was innervating one spine now innervating two spines from the same cell. Rather, estrogen induces a divergence of each bouton's output: the bouton that formerly innervated one cell now innervates at least two different cells. This result is reminiscent of developing systems where neurons early in life innervate far more target cells than they do in adulthood (13).

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What is the functional significance of these estrogenic changes in brain wiring? As usual in these formative years of behavioral neuroscience, only speculative answers are available. There is a growing sense that estrogen exerts a beneficial effect on cognitive functioning, especially in the aged brain (14). Studies of synaptic plasticity in the hippocampus, specifically the phenomenon of long-term potentiation as a model Hebbian synapse, emphasize the possible importance of synchronized input for gating changes in synaptic strength. Thus, it does not seem inconceivable that the presynaptic bouton simultaneously exciting neighboring spines from two different CA1 cells could serve to increase synaptic plasticity. Exactly how that could happen elicits little more than hand waving at present. But another possible functional consequence of the neural rewiring centers on a disadvantageous effect of estrogen. Estrogen treatment can decrease the threshold for hippocampal seizures (15). The simultaneous excitation of two different CA1 cells by the same terminal bouton in estrogen-treated animals would be expected to increase the synchronization of firing that can lead to seizure (16). Perhaps steroid analogs could be developed to counteract these seizure-facilitating effects of estrogen without interfering with the beneficial effects of the steroid.

There are plenty of other questions remaining. For example, where does estrogen act to induce these new spines and this new pattern of innervation? CA1 cells themselves are not particularly rich in estrogen receptors (17), so perhaps the steroid does not act directly on those neurons to trigger the new spines. Alternatively, estrogen might act on the neural afferents, inducing the expansion of presynaptic boutons, which draw forth new spines from the nearby postsynaptic dendrite. Also, what determines where the new spines will arise to form a ménage à trois with a preexisting bouton-spine pair? Yankova *et al.* note that the MSBs in estrogen-treated animals seem to arise in clumps along the dendrite, suggesting that some sites are more susceptible to this plasticity than others. What demarcates the plasticity-prone sites? Are they innervated by a particular class of afferents, which perhaps produce estrogen receptors? Are these same sites remodeled over and over during the cyclical synaptic changes across the estrous cycle, or do different parts of the dendrite remodel in subsequent cycles? For now, mark your scorecard to indicate another example of steroid hormones altering adult neuroanatomy, and another example of how patterns of neural connections can be reorganized in the adult brain. Instead of asking whether new synapses can come or go, perhaps now we should ask whether any particular synapse in the brain remains unchanged for more than a short while.

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