To change the brain

by

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Epitome: An exploration of the promise and limits of brain plasticity through the study of adult neurogenesis

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Most of us move through the world with a sense of self that remains fairly constant from moment to moment and day to day. We might experience passing changes in emotions or mood, and we may notice that some aspects of our personality become intensified or moderated over the years with age or experience, but our fundamental experience of living as ourselves feels mostly consistent. Yet we (at least the more optimistic among us) also feel that we can make significant changes to our lives—learn new skills, develop new relationships, or form new habits. All organisms must balance these simultaneous requirements for both stability and plasticity in order to navigate their worlds. How are these demands reflected in the brain, the organ that we now understand to be the main orchestrator of our perceptual experiences?[1]

If we could open the skull and observe the brain directly, on a large-scale level, things might seem quite stable. The large axonal tracts connecting different brain regions are formed during early development and persist throughout life. Most of the neurons, the major cells responsible for transmitting information within and between brain regions, are born during embryonic development, never to divide again (there are important exceptions, which we will examine in detail in subsequent pages). But if we were to continue to zoom in to the microscopic level, we would see a tangled web of more than a trillion synapses, the connections between neurons, many of which are constantly growing and shrinking, breaking and forming. If we could zoom in even farther, we would see proteins zipping up and down axons (output sites of neurons) and dendrites (the receiving sites) to supply changing synapses. Moreover, proteins, the building blocks of all cells, are undergoing constant turnover, some being replaced on an hourly, daily, or weekly basis to combat the wear and tear of everyday use. Clearly, the brain possesses a substrate for plasticity—it must constantly replace its constituent parts, much like the ship that is reassembled over time in the age-old thought experiment.[2] But is each piece put back into the same place or are the parts shuffled around? And perhaps most provocatively, can entirely new parts be added?

How much can the brain change? It depends.

On one hand, there are extraordinary stories of recovery after brain injuries or disease. In one compelling example, researchers have recently become aware of blind individuals who have taught themselves to use a kind of echolocation to form a mental map of their surroundings, allowing them to move through the world and even pursue activities such as hiking and mountain biking without assistance.[3] There is evidence that areas of the brain involved in visual processing may have increased activation in these people in response to sounds involved in echolocation, potentially suggesting the

strengthening of existing connections or formation of new ones in a way that allows these individuals to experience the world in a way few humans ever have before.[4]

On the other hand, there is ample research suggesting that early life experiences imprint lasting, and sometimes tragic, consequences on the brain that may be irreversible. Classic studies in the 1960s developed the concept of "critical periods," the idea that there are windows of time during which children must experience appropriate sensory inputs to set in motion normal brain development. Without this exposure, they will suffer lasting deficits. For example, if a child who has a congenital cataract that reduces vision in one eye does not have the cataract removed before about the age of seven, he or she will develop a condition called amblyopia, commonly known as a "lazy eye," preventing normal depth perception. Correcting the condition later in life does not restore normal vision, since visual areas of the brain are no longer able to reorganize inputs in the same way they could during childhood. Such sensory critical periods have been well-characterized in animal models as well as humans, but there is emerging evidence that the concept may also apply to more uniquely human experiences, such as language acquisition and mental illness. For example, a history of childhood trauma is a significant environmental risk factor for depression and other psychiatric illnesses later in life.[5]

How can we reconcile our brains' amazing capacity for adaptation with these tragic limitations? How much can the brain change in response to experience (and is the ability to adjust always desirable)? To answer these questions, we need to understand brain plasticity at a mechanistic level, from the proteins that make up neurons to the neurons that form brain circuits.

New synapses and new neurons

If you scrape your knee or break a bone, your body will produce new skin cells or bone cells to repair the wound. But what would happen if you were to experience a stroke that decimated the cells in some area of the brain? Some types of brain cells would be produced, but not neurons. Neurons are born during fetal development, never to divide again. Patients typically experience some recovery of function after a stroke, but this is thought to be due to compensation by other brain regions and the formation of new synapses between existing neurons. New neurons cannot be produced in the adult brain.

Or so the thinking went for the first one hundred years of modern neuroscience.

In the 1960s, a young biologist named Joseph Altman was studying the brain's response to an injury in rats. He was initially disappointed to see that no new neurons were produced around the injury site, but serendipitously, he noticed that cells with some of the hallmarks of neurons were indeed born,

surprisingly in regions of the brain quite distant from the injury site. Unfortunately, the techniques available at the time did not allow a conclusive demonstration that the cells were in fact neurons, and the scientific community as a whole did not accept the finding. It took another twenty years and discoveries of adult neurogenesis in animals ranging from canaries to marmosets for the field to recognize the phenomenon.[6]

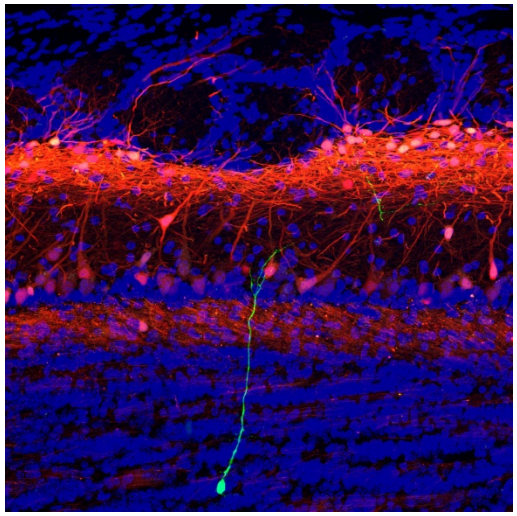

Figure 1
An adult-born neuron (green) in the olfactory bulb of a mouse must integrate into existing brain circuits (red and blue). Unpublished image by Martin Wienisch

Neurogenesis can be considered perhaps the most dramatic form of plasticity available to the adult brain. Instead of the strengthening of existing synapses or the formation of new ones (the classically studied mechanisms of brain plasticity), adult neurogenesis involves the addition of entirely new neurons to existing brain circuits. This is no small feat – these neurons must migrate through the tangled array of cell bodies, axons, and dendrites that have been present since birth (Figure 1), arrive at their final destinations, grow new dendrites, and make new synaptic connections. The process does not occur everywhere but rather is restricted, at least in mammals, to only a couple of brain regions, including the olfactory bulb (the first area involved in processing the sense of smell) and the hippocampus (involved in memory and spatial navigation). Why these two regions and really, why should the brain bother to engage in the presumably energetically intensive process of producing entirely new neurons at all, at least in the absence of some injury or disease? These are burning questions in the field to which we still don't have satisfactory answers. Many groups are trying to address these questions with variety of approaches, including inactivating new neurons to determine if they are important for certain behaviors and developing computational models to understand what additional processing power new neurons might add to the circuit. But models must be based on data, and when I began my PhD, no one had ever observed what these new neurons (henceforth called adult-born neurons, to distinguish them from the majority of neurons which are embryonically born) were doing in the intact brain. Early studies in the field quantified the morphology and synaptic connections of these cells in preserved tissue. However, it is difficult to predict from these measurements how adult-born neurons might respond to stimuli in the intact animal, *in vivo*. Fortunately, a method that could help investigate this question had already been developed and was coming into wider use just as I began my PhD.

A window into the brain

The idea of removing a piece of the skull and implanting a piece of glass in its place has been around for several decades,[7] but the technique became a mainstay of modern neuroscience in the 2010s when it began to be combined with emerging imaging technologies. In particular, two-photon microscopy is an exciting new way to image deep in the living brain, far beyond what was previously possible. The technique relies on the fact that although the brain is quite opaque to visible wavelengths of light, appearing a pinkish color to the naked eye (Figure 2), infrared wavelengths can penetrate brain tissue much more efficiently. This means that microscopes employing the latter type of light, along with a specialized laser and appropriate fluorescent molecules (which absorb and then emit light), can allow visualization of parts of the brain that would be impossible to see with a standard light microscope.

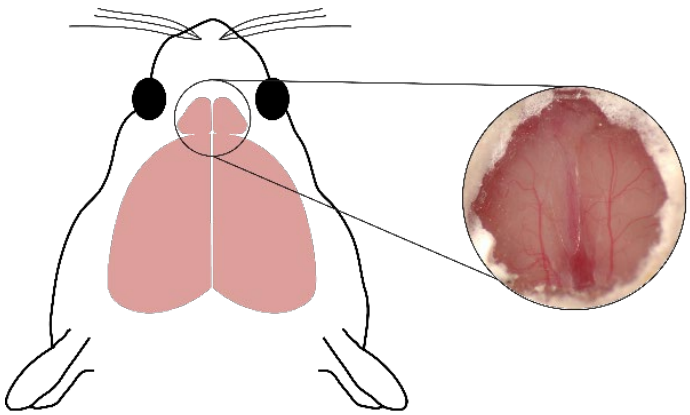

Figure 2:
Schematic showing the location of the cranial window over the olfactory bulbs at the front of a mouse's brain. Inset, view through a cranial window implanted by the author showing vasculature on the surface of the brain.

Before I could utilize this exciting imaging technology to observe adult-born neurons *in vivo*, I first had to master the surgery required to implant the cranial window. Performed under sterile conditions and utilizing many of the same anesthetics and pre-surgery drug regimens as human neurosurgery, this was no small task (or rather, it was quite small—the mouse brain is about 1/100 the size of the human brain and therefore the surgery requires special miniaturized tools, many of them adapted from dental surgery). If the surgery is performed improperly, a layer of bone often regrows under the cranial window, ruining imaging clarity. After months of frustration, I mastered the technique and was able to implant a 3 mm glass window over each mouse's pair of olfactory bulbs and secure the edges with cement. Mice quickly recover from this surgery and live out their normal lifespans, seemingly unperturbed by our newfound ability to spy on their thoughts (though of course our understanding is still rudimentary at best). Now I just needed a way to visualize the adult-born neurons specifically against the background of all the other cells in the brain.

Co-opting viruses to study neurons

Viruses are a pain in daily life, but they are a boon to neuroscientists. Using different combinations of genetic elements, viruses can deliver genes for fluorescent proteins to cell types of interest, which will then be expressed by the cells and can be visualized by microscopy. Sometimes this is accomplished with promoters with varying degrees of specificity for particular cell types, but one feature of adult neurogenesis offers an even more elegant solution—since these cells migrate from their place of origin to the olfactory bulb[8], it is possible to target a virus to their migratory route, infecting only a cohort of cells, all born on a specific day. Since only a few cells are labeled in this way (the background of millions of unlabeled cells will appear black), the cells of interest can be repeatedly located and tracked over time.

For my first set of experiments, I decided to combine these techniques—viral labeling, cranial window implantation, and two photon microscopy—to track the formation and elimination of dendritic spines, the locations where synapses are found. This would help answer the question of how adult-born neurons integrate into existing circuits—do they quickly decide on a suite of synaptic partners and maintain these relationships or do they take their time and sample all the possibilities the circuit has to offer? The answer was resounding support for the latter hypothesis. Only about 30% of the young cells' spines were stable compared to about 60% for mature cells (Figure 3).[9] This suggests that young cells sample synaptic partners at a frenzied pace as soon as they enter the circuit but this pace slows as they mature, much like a college student might try out many new friendships during her freshman year and maintain only a subset of these to form a core group of friends later on. But just as a college student's behavior might be shaped by both her friendships and her own personal values, an adult-born neuron's behavior is a property both of its synaptic connections and its intrinsic properties (its excitability, which is shaped by which charged ions it allows across its membrane and in response to which stimuli). In addition, imaging of spines cannot predict which synapses are functional and how strong they might be. Thus, visualizing a neuron's synaptic connections alone is not enough to predict

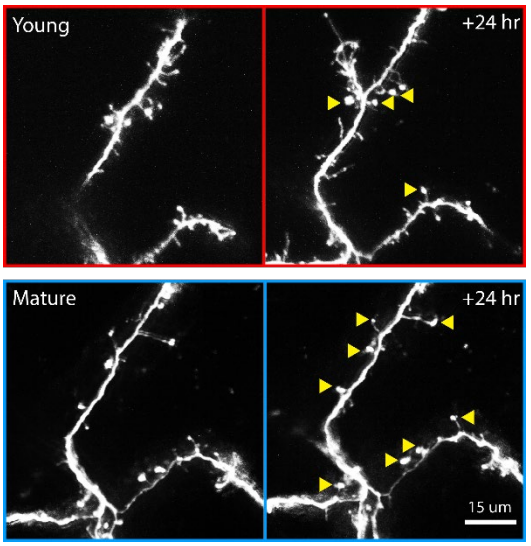

Figure 3:
Images showing the dendrites of an adult-born neuron imaged at young or mature stages on consecutive days. Stable spines are marked with a yellow arrowhead and are more abundant on the mature dendrite.

what it might be doing in the circuit. For that, we would need a method to observe the activity of adult-born neurons.

What does neural activity look like?

Asked this question, many classical neuroscientists might envision an oscilloscope displaying traces of electrical activity. That's because neurons use electrical signals called action potentials to communicate and for decades neuroscientists have recorded these traces of neural activity. But electrical activity is blind—there is no way to identify which neurons are the source of these signals nor to record the same neuron over time,[10] both of which are necessary to understand the activity of adult-born neurons. Fortunately, in parallel with the development of two-photon imaging, many groups spent the last decade creating and improving ways to make the electrical activity of neurons visible to microscopy. These methods rely on the fact that neurons at rest typically have very low concentrations of calcium, but when the neuron fires an action potential, this electrical activity is coupled with a sharp increase in intracellular calcium. In what I view as one of the most elegant feats of modern bioengineering, scientists have developed optical sensors of calcium, the most widespread of which is called GCaMP[11,12]. This amazing protein is based on the popular green fluorescent protein (GFP) but with a twist. Standard GFP has a barrel-shaped structure with the light-absorbing part (the chromophore) in the center, shielded from the environment by the barrel. To make GFP fluorescence dependent on calcium, researchers broke the barrel, exposing the chromophore to the aqueous environment of the cell and dampening its fluorescence. They then added a calcium-binding domain to one side of the barrel and its binding partner to the other. In a true triumph of protein engineering, when calcium is present, these new pieces act like a hinge to close the barrel and once again protect the chromophore and allow fluorescence.

These innovations mean that I could make a virus encoding GCaMP, inject it into a mouse's brain to target migrating adult-born neurons, and use two-photon microscopy to visualize these cells' activity *in vivo* as flashes of light.

Twinkle, twinkle little neuron

Holed up in the dark basement room where the lab's two-photon microscope is located (the experiment must be shielded from ambient light, which could be picked up by the highly sensitive instruments and thereby increase the background noise in the images), I felt nervous before every

experimental imaging session. I have always felt ethically conflicted about using animals in research, and though these mice were given as much veterinary care as any pet undergoing surgery, there was no getting around the fact that this was for the pursuit of basic science and not for their own health. And so I wondered: Had I injected the virus in exactly the right place? Would the cranial window have good imaging quality? Would the cells express high enough levels of GCaMP for me to see it? With my heart racing, I put each mouse under the microscope, one by one. Nervously adjusting the laser power and scanning different areas under the cranial window, I breathed a sigh of relief as soon as I found the telltale spiderweb of dendrites that signaled success (Figure 4). I then used a system of rewards[13] to train mice to sit still under the microscope so that I could image the responses of adult-born neurons expressing GCaMP while the mice smelled a panel of different odor molecules (Figure 5). Because the patterns of dendritic arbors were so distinctive, I could also track the same neurons over time, imaging the very same cells week after week as they matured and the mouse went about its business in between imaging sessions.

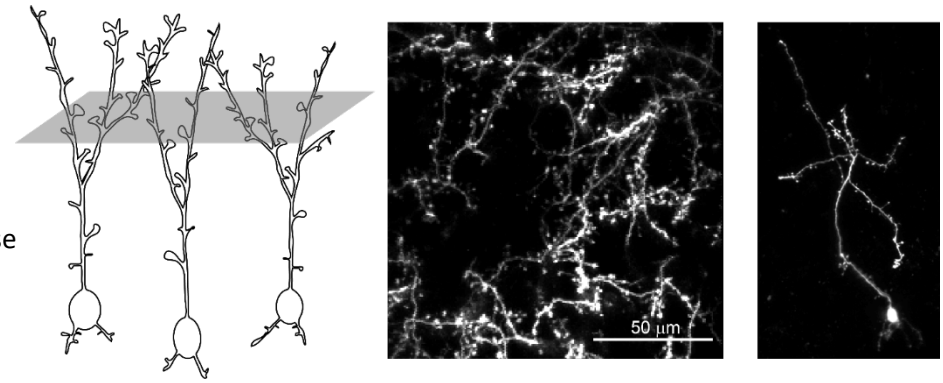

Figure 4:
Left, Schematic showing the three-dimensional structure of the neurons imaged, with the cell bodies located deeper in the brain and the dendrites extending toward the surface.
Center, Example image showing the dendrites of adult-born neurons labeled with a virus in a living mouse. The image was taken as a single plane through the dendrites, as depicted in the schematic.
Right, Three-dimensional reconstruction of many single plane images to show the entire structure of an adult-born neuron.

Scientific discoveries are often described as a single moment of insight, a moment when everything suddenly becomes clear. Perhaps this applies more to theoretical insights than experimental data, however, because experiments rely on obtaining

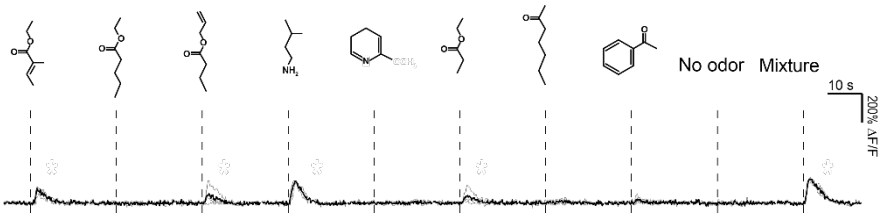

Figure 5:
Example GCaMP responses of a single dendrite to different odor molecules. Each trial is shown in light gray, and the average is in black. The responses are plotted as ΔF/F, which compares the change in GCaMP fluorescence during the response period to the baseline before the odor comes on.

sufficient sample sizes to draw statistical conclusions. Though I do remember the first moment of excitedly seeing the GCaMP light up under the microscope, bright enough that I could see the cells' activity even before analyzing it, it took a few hundreds of cells in a couple dozen mice to prove a result. Even still, those first moments of being the first to observe something (as far as one knows) are among the sweetest rewards science can offer.

Though this experiment was intrinsically open-ended, my colleagues and I had a couple of hypotheses about what we might find (Figure 6). We expected that early on, when the new neurons had not yet had time to make extensive synaptic connections, they would be activated by only a few stimuli and that they might begin to respond to more stimuli as they integrated into the network. This would likely plateau (Figure 6, Hypothesis 1) and perhaps eventually decrease (Figure 6, Hypothesis 2) as some connections were pruned away. Instead, we found nearly the opposite—the cells responded to many odors already in the first imaging session, and some of these responses were lost as they matured, resulting in increased selectivity for particular stimuli. In addition, we found that this process was sensitive to sensory experiences: we could prolong it by exposing the mice to many new odors in their home cages. After long months spent performing the required control experiments (we had to demonstrate that the decrease in the cells' responsiveness was not simply due to changes in the brain after the cranial window was implanted that resolved over time and that the prolonged expression of GCaMP was not damaging the cells), we were confident of the results.[14] These experiments made us the first to observe the functional development of individual adult-born neurons in the adult brain and suggested that these cells each have their own critical periods, much like those during early brain development. Combined with my earlier results showing a high degree of spine formation and elimination in young cells, I began to form a hypothesis that these young neurons were entering the circuit and spending their critical periods sampling the activity of the existing cells and searching for the "right" synaptic partners. To test this hypothesis would require a whole new suite of experimental skills.

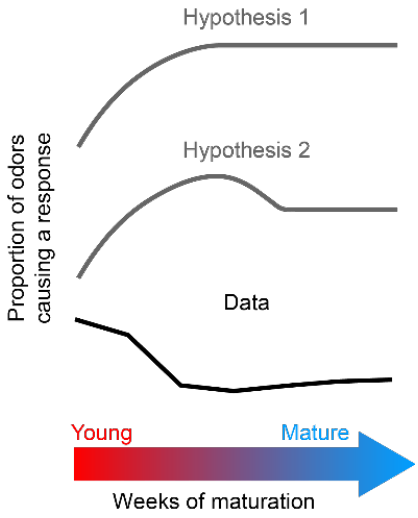

Figure 6:
Above, possible hypotheses about how adult-born neurons' responses to odors might change over development.
Below, actual data showing the average across all cells recorded for seven weeks.

Going back to basics

As exciting as it was to use two-photon imaging to observe neural activity in a living organism as it experienced its environment, this technique (at least for now)[15] is still limited—I could only observe the cells that I had labeled with the virus, but I had no way to visualize their synaptic partners. For that, I would have to go back to more traditional methods for recording electrical activity.

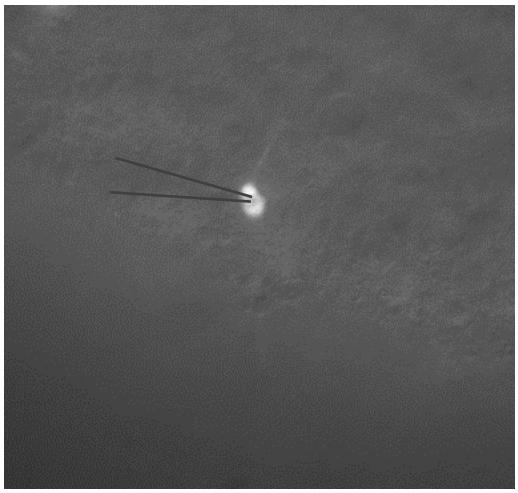

Figure 7:
View through a microscope of the first step of patch clamp recording from a fluorescent adult-born neuron (the gray background consists of many other unlabeled cells), which involves sealing a glass pipet to the cell's membrane. The glass pipet is outlined to make it more visible.

Patch clamp recording relies on the serendipitous fact that glass is very sticky to the membranes that encase living cells.[16] When a glass pipet is placed in just the right spot on a living cell and gentle suction is applied to pull a bit of the membrane into the pipet, a tight seal between the glass and the membrane forms (Figure 7). The experimenter must apply another quick burst of suction (the setup usually involves a long plastic tube affixed to the glass pipet, and the suction is applied with the mouth--novices are instructed to make a loud kissing sound at this last step) to break the cell's membrane, allowing the pipet, which contains a recording electrode, to access the inside of the cell. This remarkable setup allows the experimenter to record currents or voltages in real time that result from activating the synaptic inputs to this particular cell.

Though the patch clamp technique has been around for nearly fifty years,[17] recent developments have opened new opportunities for its use. Traditionally, the technique could be used in concert with electrical stimulation of the inputs to the recorded cell, but this is somewhat imprecise and only works well for inputs that are bundled together and located distant from the recording site (otherwise the stimulation may directly affect the cell of interest). Fortunately, I already had experience in implementing a new technique that allows more precise activation of particular cell types than ever before.

How can algae help us understand the brain?

I first became aware of the technique known as optogenetics when my high school biology teacher had the class read a New York Times article describing it.[18] I found the idea so exciting that I

posted the article on my bulletin board, where it remained until I left for college. In my sophomore year at Stanford University, as I was considering which lab to choose for my honors thesis work, I remembered that one of the scientists mentioned in the article, Karl Deisseroth, was a professor there.

Several years before I began my undergraduate degree, Karl Deisseroth, along with Ed Boyden and Feng Zhang, were the first to demonstrate a simple optogenetic system that worked in mammalian neurons and subsequently put significant effort toward improving and developing it for widespread use in the neuroscience community.[19] The technique hinges on the fact that neurons normally become activated when channels open that allow positively charged sodium ions into the cell. This typically happens in response to synaptic activity, but Deisseroth, Boyden, and Zhang put the genes for a channel called channelrhodopsin from green algae into mammalian neurons. This remarkable channel opens in response to blue light and allows positively charged ions to enter cells, directly activating them. Since then, they, along with many other groups, have engineered versions of channelrhodopsin that respond to different colors of light, or have different rates of activation, or that allow negatively charged ions into the cell to suppress activity, leading to a whole new suite of tools to manipulate neuronal activity with more genetic and temporal precision than ever before.

Optogenetics has many applications in behaving animals—in something that seems straight out of science fiction, during my time as an undergraduate in the Deisseroth lab, I used a fiber to deliver light to specific parts of a mouse's brain and watched its behavior change in real time as I switched the light on or off. These types of experiments can help us understand which cell types in which parts of the brain are involved in different activities. But optogenetics is also incredibly useful for dissecting neural circuits and probing which neurons are synaptically connected, and it was this application that I had in mind as I continued my efforts to understand how new neurons could find the right connections as they developed in an adult brain.

Flipping the light switch

The experiment was conceptually simple: record from individual adult-born neurons while sequentially activating different types of presynaptic cells, which had been infected by a virus to genetically engineer them to express channelrhodopsin, with light. Simple in theory does not always mean simple in practice, however. These experiments require hours of careful attention: the tissue slices must be prepared quickly to preserve the cells' viability (which usually lasts about 8 hours, maximum), the solutions used for recording must be meticulously formulated to mimic the brain's natural environment, and all of the recording equipment must be thoroughly shielded from electrical

noise, among many other factors. Even an expert will not be able to obtain satisfactory recordings from every targeted cell, and this lends a quality of gambling to the experiment, albeit requiring a higher ratio of skill to luck. Every time the membrane of a cell is satisfactorily broken, the electrical trace jumps into the recording configuration (Figure 8), and I confess sometimes I hop a little in my chair with it.

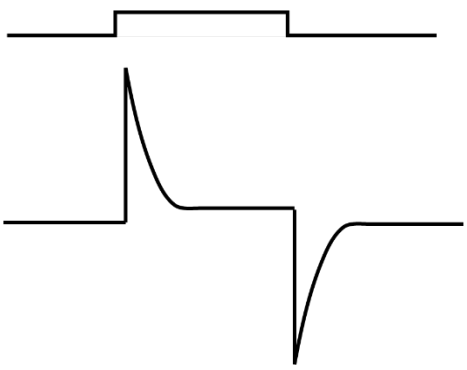

Figure 8:
Above, drawing of an electrical trace in response to a test voltage pulse recorded after a glass pipet is sealed onto a living cell
Below, drawing of a recording in response to the same voltage pulse after the cell's membrane has been successfully broken. The change in shape of the recording is due to the electrical properties of the cell under investigation.

After hours of recording, at the height of these experiments, I would see my cells everywhere—a full moon behind a thin veil of clouds looked just like a labeled neuron nestled deep in a tissue slice, the water droplets coursing down the walls of my shower appeared like thin dendrites, as I followed their progress by focusing up or down through the microscope. The obsession was worth it—after recording dozens, perhaps hundreds, of cells, I began to form a picture of what could be going on.

The young cells had fewer synapses, as expected since they were still at an early stage of development. How then, could I explain the result that in the living animal, they responded to so many more stimuli than the mature cells? A simple hypothesis is just that they are more excitable. However, when I injected current into the cells to elicit action potential firing, the young cells actually fired fewer action potentials than the mature cells, arguing that this hypothesis was incorrect. So I was back to considering if something could be different about the synapses in the young cells.

I spent nearly a year testing all of the different types of synaptic inputs to the adult-born neurons (using viruses to target different cell types and light to activate each in turn) to understand how the synapses develop and if they change as the cells mature, but I came up empty. Although I catalogued some interesting features of synaptic development, I could find no differences in synapses that could account for the higher responses of the young cells to odor stimuli. After all this work, it was a conversation with a colleague that finally helped me to think "outside the synapse," and develop a new hypothesis.

One intriguing possibility is that young adult-born neurons may have receptors located outside of typical synapses that could still bind to neurotransmitter and cause a response. This could allow them listen in to nearby synapses, but likely only at times where many nearby synapses were activated at

once. These receptors would be invisible to us with standard assays of synapse numbers, since this is normally assessed in conditions where few presynaptic neurons are active, which is not representative of the concerted activity that would occur in the living brain as a mouse smells an odor. As I write this, mice expressing channelrhodopsin in all of the main excitatory neurons in the olfactory bulb are breeding in our department's animal facility, and their offspring will allow me to test this hypothesis.[20] If true, this result would suggest a new mechanism for adult-born neurons to sense the activity of existing brain networks, which might help them target their connections to the most active cells and position them to enhance plasticity.

Though we are just at the beginning of understanding mechanistically how adult neurogenesis contributes to brain circuits, there is already evidence that it may be important for learning. In songbirds, new neurons are incorporated into brain regions involved in controlling song production, and this is correlated with the seasonal learning of a new song. If these neurons are ablated, the ability to sing is disrupted. In mice, neurogenesis appears to be involved in learning and remembering spatial locations or odor stimuli that are similar, yet distinct, and distinguishing them later. We also know that exercise and environmental enrichment (i.e. mental engagement with new experiences) stimulate more adult-born neurons to be produced, potentially providing some actionable strategies for those of us wishing to stimulate the process within our own brains. But is this even possible? Do the lessons learned from mice apply to humans?

Does Adult Neurogenesis Occur in the Human Brain?

The fact that I recently attended a conference session with this title immediately gives away the answer: we still aren't sure. In a packed room at the annual Society for Neuroscience meeting in Chicago, two of the leading researchers in the field (who had each just published papers presenting data on opposite sides of the argument[21]) disagreed with each other, albeit quite politely. The one point that everyone in the room could appreciate is that it is very difficult to study this process in humans; after all, this is why we use mice as a model organism. We cannot inject viruses or implant cranial windows in human subjects. One pioneering study[22], already two decades old, used another method that is often applied in mouse studies, injecting a compound into human cancer patients called bromodeoxyuridine, which becomes part of a cell's DNA, but only in cells that are actively dividing, thus labeling only neurons that were born at the time of the injection. This study did claim to find adult-born neurons in humans, albeit not in the olfactory bulb but in a different region, the hippocampus. However, the number of patients was small and this study can never be replicated because bromodeoxyuridine is no longer given

to cancer patients (and it would be unethical to give a DNA-altering drug to healthy people). So we are left with less direct methods (typically the goal is to find reliable markers for immature cells) of searching for newly born neurons in the haystack of existing cells, dendrites, and axons in the human brain.

Though it is certainly important that we make progress towards this basic understanding, in one sense it may not matter if the healthy human brain normally produces new neurons: perhaps we could put them there ourselves. I recently had lunch with Magdalena Götz, a German neuroscientist who has made significant progress towards this goal. The idea goes all the way back to the original discovery of adult neurogenesis. Recall that Joseph Altman was initially looking for new neurons to be produced around a site of injury. Though new neurons were not found near the injury site, he did observe that another cell type was produced in abundance. These cells, called astrocytes after their star-like morphology (Figure 9), are not directly involved in signaling but normally support neuronal functions by cleaning up excess signaling molecules and regulating blood flow. Götz's insight was that astrocytes are closely related to the brain's stem cells, the cells that divide during embryonic development to produce all of the other cell types in the brain. Her lab developed a method to use a virus to infect astrocytes after an injury with factors that could turn back the clock and transform them back into their stem cell ancestors. Remarkably, these stem cells then began to generate multiple types of new neurons—the same types that were damaged by the injury. These neurons, similar to the adult-born neurons that I studied, seemed to undergo a process of activity-dependent synaptic development and eventually made connections with appropriate synaptic partners (although the question of whether they exhibit normal activity patterns and responses to stimuli remains to be studied).[23] Although this strategy has only been applied in mice to date, a simpler (but less versatile) strategy is already underway in human patients[24] to implant stem cells that could replace dopaminergic neurons, the cell type that degenerates in patients with Parkinson's disease. These studies raise the prospect that progress in stem cell biology, combined with a mechanistic understanding of how new cells can integrate into existing brain circuits, could offer

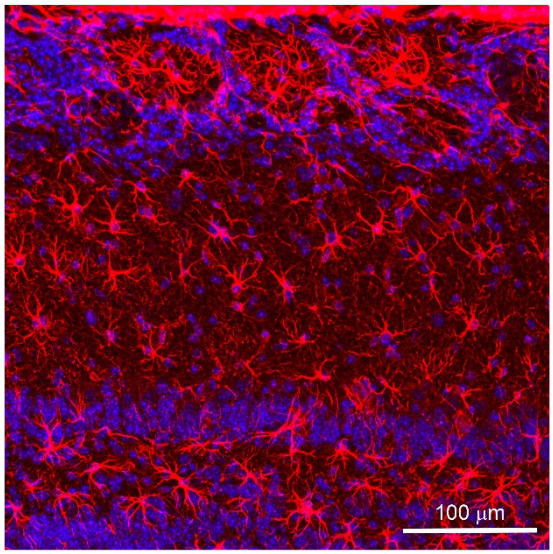

Figure 9:
Astrocytes (red) are stelliform cells whose extensive processes enwrap synapses and blood vessels to act to support neuronal functions. Cell nuclei are stained blue in this image to show the location of cell bodies.

new strategies for repairing the brain in the case of traumatic brain injuries and neurodegenerative disorders.

Aside from these important possible medical applications, there is yet another reason to continue the study of adult neurogenesis. Salamanders can regenerate nearly a quarter of their entire brains in response to an injury and fish have ongoing neurogenesis in dozens of brain regions as their brain grows throughout their lifespan. Why don't we? We must place adult neurogenesis, and indeed brain plasticity in general, in an evolutionary framework if we are ever to understand the balance or perhaps the tradeoff between plasticity and stability. As our lineage diverged from that of other primates, did the human brain sacrifice plasticity to grow more powerful or was this simply an evolutionary accident, perhaps one that could be remedied using the strategies described above? Or does the human ability to integrate and remember past experiences over our long lifespans require more stability and enforce more rigidity in our neural circuits, at the expense of potential for change? Perhaps the answers will give us a greater appreciation for our connection to the rest of the natural world and the place of our brains within it.

[1] The Greek physician Galen of Pergamon in the first century AD was one of the first to recognize the brain as the location where information from the senses is integrated and actions are planned, in contrast to several other ancient scholars who believed the heart was the seat of intelligence.

[2] This philosophical thought experiment (the Ship of Theseus) asks whether something (such as the famous ship sailed by the Greek hero Theseus) that has had all its individual parts replaced is still the same entity.

[3] Kremer, William (12 September 2012). "Human echolocation: Using tongue-clicks to navigate the world". BBC. Retrieved November 1, 2019.

[4] The following is a review describing this emerging field:

Thaler L, Goodale MA. 2016. Echolocation in humans: an overview. Wiley Interdiscip Rev Cogn Sci 7:382–393. doi:10.1002/wcs.1408

[5] For a review of this complex literature, see the following:
Carr, C. P., Martins, C. M. S., Stingel, A. M., Lemgruber, V. B., & Juruena, M. F. (2013). The role of early life stress in adult psychiatric disorders: A systematic review according to childhood trauma subtypes. *Journal of Nervous and Mental Disease*, 201(12), 1007–1020. doi:10.1097/NMD.0000000000000049

[6] This illuminating chapter in the history of neuroscience is described in an excellent article:
Specter, Michael. “Rethinking the Brain.” *The New Yorker*, 23 July 2001, pp. 42–53.

[7] One of the earliest publications describing this technique applied to studying blood flow in the brain is:

Levasseur JE, Wei EP, Raper JA, Kontos HA, Patterson JL. 1975. Detailed description of a cranial window technique for acute and chronic experiments. *Stroke* 6:308–317. doi:10.1161/01.STR.6.3.308

[8] The stem cells responsible for adult neurogenesis reside deep in a region in the middle of the brain called the subventricular zone and when they divide, their progeny must migrate several millimeters (about half the length of the entire mouse brain) to the olfactory bulb.

[9] These numbers are from the following reference and are consistent with my observations (which remained preliminary since I began to follow other research directions after this work was presented):

Sailor KA, Valley MT, Wiechert MT, Riecke H, Sun GJ, Adams W, Dennis JC, Sharafi S, Ming G, Song H, Lledo P-M. 2016. Persistent Structural Plasticity Optimizes Sensory Information Processing in the Olfactory Bulb. *Neuron* 91:384–396. doi:10.1016/j.neuron.2016.06.004

[10] There are some groups making strides in this direction, although the methods are not as direct as tracking neurons using imaging and have their own caveats.

See the following for a method to determine the identity of neurons using electrical recordings:

Lima SQ, Hromádka T, Znamenskiy P, Zador AM. 2009. PINP: A new method of tagging neuronal populations for identification during in vivo electrophysiological recording. *PLoS One* 4. doi:10.1371/journal.pone.0006099

The following reference describes a method for tracking neurons’ activity over time using electrical recordings:

Dhawale AK, Poddar R, Wolff SBE, Normand VA, Kopelowitz E, Ölveczky BP. 2017. Automated long-Term recording and analysis of neural activity in behaving animals. *Elife* 6:1–40. doi:10.7554/eLife.27702

[11] The first version in the GCaMP family of sensors is described here:

Nakai J, Ohkura M, Imoto K. 2001. A high signal-to-noise ca2+ probe composed of a single green fluorescent protein. *Nat Biotechnology* 19:137–141. doi:10.1038/84397

The version used in all my experiments was GCaMP6s, which is described here:

Chen, T., Wardill, T. J., Sun, Y., Pulver, S. R., Renninger, S. L., Baohan, A., … Kim, D. S. (2013). Ultrasensitive fluorescent proteins for imaging neuronal activity. *Nature*, *499*, 295–300. doi:10.1038/nature12354

[12] The word "GCaMP" is a good example of biologists' fondness for loosely based acronyms, combining references to the three elements of the GCaMP protein: GFP, calmodulin (the calcium binding protein), and myosin light chain kinase (a small piece of which is the part that binds to calmodulin to close the barrel of GFP).

[13] Thanks to a tip from a colleague that mice behave well when rewarded with cheese-flavored Goldfish crackers.

[14] The paper describing these results is now published:

Wallace JL, Wienisch M, Murthy VN. 2017. Development and Refinement of Functional Properties of Adult-Born Neurons. *Neuron* 96:883–896. doi:10.1016/j.neuron.2017.09.039

[15] Some approaches for these types of experiments with two photon imaging have been developed, including viruses that cross synapses and express GCaMP in the presynaptic cells (with the caveats that these viruses can be toxic to the cells and may not cross all synapses with equal efficiency):

Yonehara K, Farrow K, Ghanem A, Hillier D, Balint K, Teixeira M, Jüttner J, Noda M, Neve R, Conzelmann KK, Roska B. 2013. The first stage of cardinal direction selectivity is localized to the dendrites of retinal ganglion cells. *Neuron* 79:1078–1085. doi:10.1016/j.neuron.2013.08.005

Another family of methods described in the following review rely on the ability to activate presynaptic cells while observing responses in the cells of interest (with the caveat that this is only easy to implement for presynaptic cells that are very close to the cells of interest, rather than projecting from an entirely different brain region):

Emiliani V, Cohen AE, Deisseroth K, Häusser M. 2015. All-optical interrogation of neural circuits. *Journal of Neuroscience* 35:13917–13926. doi:10.1523/JNEUROSCI.2916-15.2015

[16] This only works, however, if the glass is very clean, an endless source of frustration for electrophysiologists who must be constantly vigilant against dust or other debris.

[17] It was developed by Erwin Neher and Bert Sakmann in the 1970s and 1980s, and they shared a Nobel Prize in Physiology or Medicine for the work in 1991.

[18] The article was:

Chen, Ingfei. "The Beam of Light That Flips a Switch That Turns on the Brain." *New York Times*, 14 Aug. 2007.

[19] Though these three generally receive the lion's share of the credit for the development of the technique, they were not the first to propose the idea nor the first to implement it. Francis Crick of DNA fame is generally recognized as the first to suggest the idea in a lecture at the University of California in San Diego in 1999. Three years later, Boris Zemelman and Gero Miesenböck were the first to employ a genetic method to control neurons with light, but they used a fruit fly receptor protein, which is quite different from the algal channels in wide use today. An engaging history of the discovery process written by one of the major players, Ed Boyden, is here:

Boyden ES. 2011. A history of optogenetics: The development of tools for controlling brain circuits with light. *F1000 Biology Reports* 3:1–12. doi:10.3410/B3-11

[20] Virally expressed channelrhodopsin is not ideal for this experiment since viruses only infect a subset of the neurons and the number might be variable from mouse to mouse, so in this case we used a line of mice genetically engineered to express the channel.

[21] The papers from the two groups are:

(1) Sorrells SF, Paredes MF, Cebrian-Silla A, Sandoval K, Qi D, Kelley KW, James D, Mayer S, Chang J, Auguste KI, Chang EF, Gutierrez AJ, Kriegstein AR, Mathern GW, Oldham MC, Huang EJ, Garcia-Verdugo JM, Yang Z, Alvarez-Buylla A. 2018. Human hippocampal neurogenesis drops sharply in children to undetectable levels in adults. *Nature*. doi:10.1038/nature25975

(2) Boldrini M, Fulmore CA, Tartt AN, Simeon LR, Pavlova I, Poposka V, Rosoklija GB, Stankov A, Arango V, Dwork AJ, Hen R, Mann JJ. 2018. Human Hippocampal Neurogenesis Persists throughout Aging. *Cell Stem Cell* 22:589-599.e5. doi:10.1016/j.stem.2018.03.015

[22] Riksson PESE, Erfilieva EKP, Riksson THBJ, Lborn ANNAA, Ordborg CLN, Eterson DAAP, Gage F. 1998. Neurogenesis in the adult human hippocampus. *Nature Medicine* 4:1313–1317.

[23] Mattugini, N., Bocchi, R., Scheuss, V., Russo, G. L., Torper, O., Lao, C. L., & Gotz, M. (2019). Inducing Different Neuronal Subtypes from Astrocytes in the Injured Mouse Cerebral Cortex Article Inducing Different Neuronal Subtypes from Astrocytes in the Injured Mouse Cerebral Cortex. *Neuron*, *103*, 1–10. https://doi.org/10.1016/j.neuron.2019.08.009

[24] Cyranoski, David. "'Reprogrammed' Stem Cells Implanted Into Patient with Parkinson's Disease." Nature News, *Nature*, 14 Nov. 2018, https://www.nature.com/articles/d41586-018-07407-9.