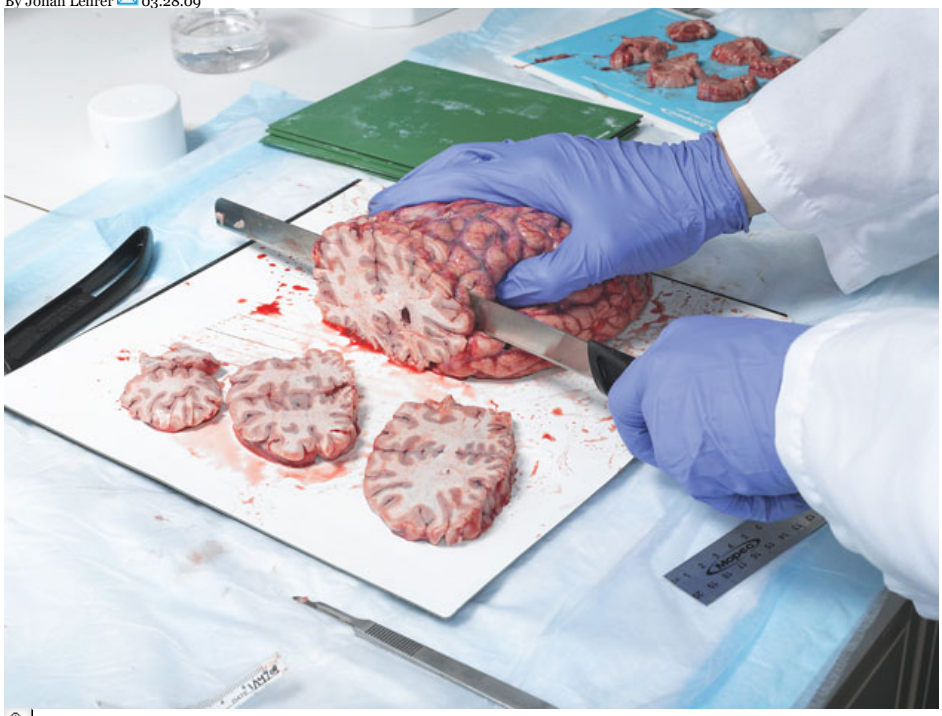
WIRED MAGAZINE: 17.04

Med-Tech : Health

Scientists Map the Brain, Gene by Gene

By Jonah Lehrer 03.28.09



"The brain is details on top of details on top of details." — Michael Hawrylycz
Photo: David Clugston

Gallery

Creating an Atlas of the Human Mind

The human brain is surprisingly bloody. I've worked in neuroscience labs, and I'm used to seeing brains that are stored in glass jars filled with formaldehyde, the preserved tissue a lifeless gray. But this brain—removed from a warm body just a few hours ago—looks bruised, its folds stained purple. Blood drips from the severed stem, forming puddles on the stainless steel table.

I'm in the dissection room of the Allen Institute for Brain Science in Seattle, and the scientist next to me is in a hurry: His specimen—this fragile cortex—is falling apart. Dying, the gray matter turns acidic and begins to eat away at itself; nucleic acids unravel, cell membranes dissolve. He takes a thin, sterilized knife and slices into the tissue with disconcerting ease. I'm reminded of Jell-O and guillotines and the meat counter at the supermarket. He saws repeatedly until the brain is reduced to a series of thin slabs, which are then photographed and rushed to a freezer. All that remains is a pool of blood, like the scene of a crime.

Behind all the gore there's a profound purpose: The scientists here are mapping the brain. And while conventional brain maps describe distinct anatomical areas, like the frontal lobes and the hippocampus—many of which were first outlined in the 19th century—the Allen Brain Atlas seeks to describe the cortex at the level of specific genes and individual neurons. Slices of tissue containing billions of brain cells will be analyzed to see which snippets of DNA are turned on in each cell.

If the institute succeeds, its maps will help scientists decipher the function of the thousands of genes that help produce the human brain. (Although the Human Genome Project was completed more than five years ago, scientists still have little idea which genes are used to make the brain, let alone where in the brain they are expressed.) For the first time, it will be possible to understand how such a complex object is assembled from a basic four-letter code.

"The maps of the brain we currently have are like those antique maps people used to draw of the New World," says Allan Jones, chief scientific officer at the Allen Institute. "We can see the crude outlines of the structure, but we have no idea what's happening on the inside." Jones is in charge of making sure the atlas gets finished. He wears starched button-up shirts and crisply pleated khakis, and he looks like the kind of guy who has a drawer full of bow ties. "Studying the brain now is like trying to navigate a vast city without any driving instructions," he says. "You don't know where you are, and you have no idea how to find what you're looking for."

Blocked Plug-in

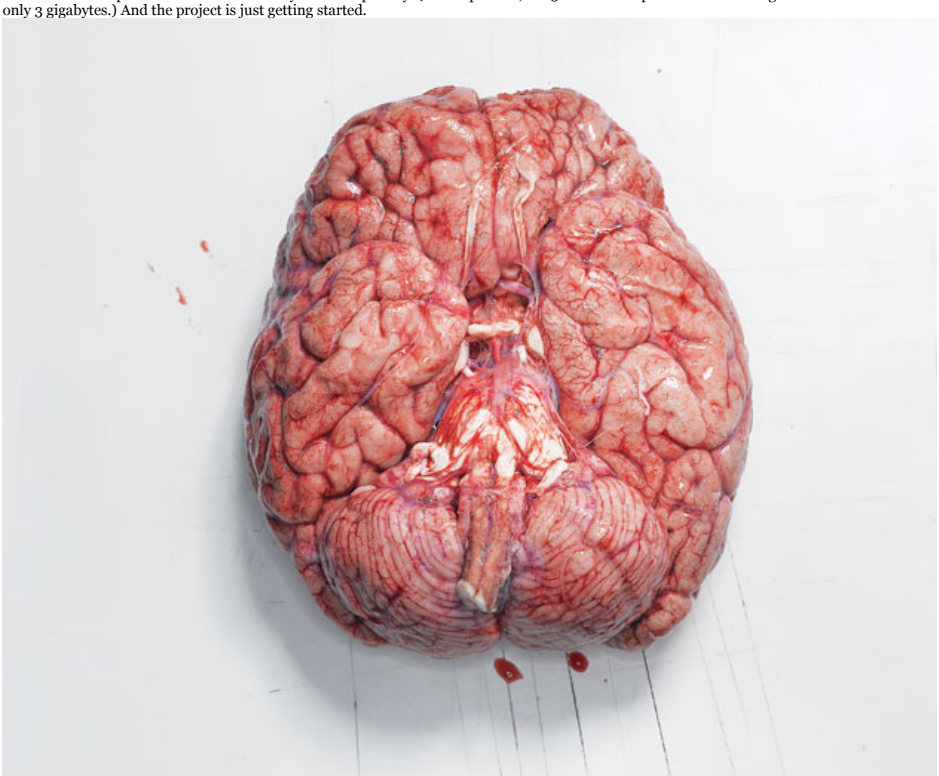
Author Jonah Lehrer spoke at San Francisco's Commonwealth Club on February 19, 2009 about the black box of the human mind.
For more from FORA.tv, visit wired.com/video.

When the project is completed in 2012, at an expected cost of $55 million, its data sets will list the roughly 20,000 genes that, switched on in the exact right place at the exact right time, give rise to this self-aware tangle of neurons. And because the vast majority of mental illnesses and disorders, from schizophrenia to autism, have a significant genetic component, scientists at the institute hope that the atlas will eventually lead to new methods of diagnosis and more effective medical treatments. To map the brain is to map its afflictions.

This enterprise is unique in one other respect: scale. "People ask me why we didn't start with a more modest goal, like trying to map some small brain area," Jones says. "The point of doing the whole brain, though, is that it allows us to really develop theories about how the brain works. Sometimes, the only way to make sense of a complex system is to be systematic."

To achieve this, the Allen Institute reimagined the scientific process. There was no grand hypothesis, or even a semblance of theory. The researchers just wanted the data, and, given the amount needed, it quickly became apparent that the work couldn't be done by hand. So, shortly after the institute was founded in 2003, Jones and his team started thinking about how to industrialize the experimental process. While modern science remains, for the most part, a field of artisans—scientists performing their own experiments at their own benches—the atlas required a high-throughput model, in which everything would be done on an efficient assembly line. Thanks to a team of new laboratory robots, what would have taken a thousand technicians several years can now be accomplished in less than 20 months.

The institute can produce more than a terabyte of data per day. (In comparison, the 3 billion base pairs in the human genome can fit in a text file that's only 3 gigabytes.) And the project is just getting started.



Preparing a fresh specimen for analysis.
Photo: David Clugston

In March 2002, Paul Allen—cofounder of Microsoft and 41st-richest person in the world—brought together a dozen neuroscientists for a three-day meeting aboard his 300-foot yacht, Tatoosh, which was anchored in Nassau, Bahamas. At the time, Allen's philanthropic work consisted of an eclectic (some say frivolous) set of endeavors. There was the Experience Music Project in Seattle, a rock-and-roll museum designed by Frank Gehry; the Allen Telescope Array, 350 radio telescopes dedicated to deep-space observation and the search for extraterrestrial life; and SpaceShipOne, the first privately funded plane developed to put a human in space. But Allen was eager to start something new: a project involving neuroscience. He was excited by the sheer uncharted mystery of the mind—one of the last, great scientific frontiers—hoping a single large-scale endeavor could transform the field.

"I first got interested in the brain through computers," Allen says. "There's a long history of artificial intelligence programs that try to mimic what the brain is doing, but they've all fallen short. Here's this incredible computer, a really astonishing piece of engineering, and we have no idea how it works."

Over several days, Allen asked the neuroscientists to imagine a way to move their field forward dramatically. "I wanted them to think big," he says. "Like the Human Genome Project, only for the brain." Some advocated focusing on a single disease, like Alzheimer's. Others argued for more investment in brain imaging technology. But a consensus emerged that what neuroscience most needed was a map, a vast atlas of gene expression that would reconcile the field's disparate experimental approaches. It's not that scientists don't know a lot about the brain—it's that they have no idea how it all fits together.

Today, you can measure the electrical activity of individual neurons, which involves plunging a microelectrode into the tissue and hoping to find an interesting cell. You can image the brain in an fMRI machine and isolate the areas that are active during certain types of mental activity. Or you can use the tools of molecular biology and study specific kinase enzymes, synaptic proteins, or RNA splices.

The problem with this multiplicity of techniques is that they fail to explain how the brain's essential elements—the wet stuff, the genetic text, the electric loom of cells—conspire to create a sentient piece of matter. Allen decided that what neuroscience needed was a tool to help get beyond these obsolete boundaries. "It became apparent to me that there were lots of scientists studying their own little area of brain, pursuing these very specific questions," he says. "But I wanted to develop something that would focus on making these crosscutting connections, so that everybody in the field could benefit."

Say, for instance, someone is investigating the anatomy of autism. The scientist has done an fMRI study that reveals abnormalities in a cortical area in autistic subjects—a bit of brain is not functioning properly—and this might help explain the symptoms of the disease. But now what? The problem has been isolated, but at a very abstract level. The research has hit a dead end.

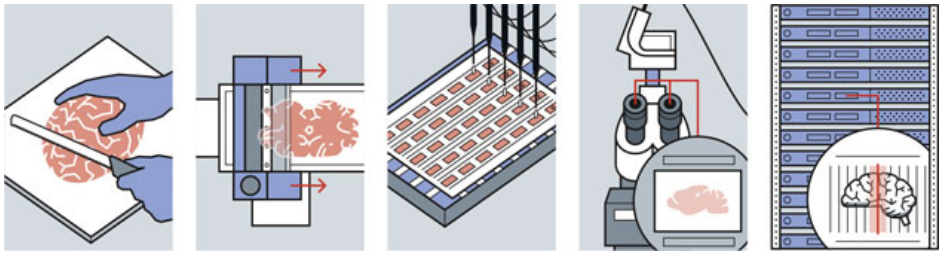
Meanwhile, another scientist is looking at autism from a very different perspective, conducting large-scale genetic studies that identify a few of the fragments of DNA associated with the disease. (Autism is one of the most heritable psychiatric disorders.) The problem with these efforts is that they often highlight obscure genes that haven't been studied. Nobody knows what these genes do, or whether they're even expressed in the brain. As a result, the research stalls and it remains completely unclear how this genetic defect might lead to the particular problems seen in the fMRIs.

But now imagine that this scientist has access to the Allen atlas. By looking at the map, he should be able to quickly see whether any of the genes known to be associated with autism—several have already been identified—are expressed in the brain areas that appear abnormal in the fMRI scans. This means that the disease can be pinpointed at a very precise level, reduced to a few dysfunctional circuits expressing the wrong set of genes. "That's what having a huge database lets you do," Allen says. "It becomes a tool that will really accelerate the pace of research." Such a map can also help neuroscientists better target their genetic searches. Instead of looking at every gene expressed in the brain—according to the institute's research, that may include nearly 80 percent of the human genome—they can focus only on those that are present in the relevant brain areas.

Then there's the mystery of the developing brain. How does something so complex manage to build itself? The Allen Institute is also measuring genetic expression in the mouse brain, from embryo to adult, to explore how the orchestra of genes is switched on and off in different areas during development. Which snippets of DNA transform the hippocampus into a center of long-term memory? Which make the amygdala a warehouse of fear and anxiety? "One of the things I've come to appreciate about the brain is the importance of location," Allen says. "It's not just a set of interchangeable parts that you can swap in and out. These brain areas are all so distinct, and for reasons we can finally begin to understand."

Industrial-Strength Science

To create a complete genetic map of the brain, scientists at the Allen Institute had to invent a high-throughput system that can process tissue and data on an unprecedented scale. Traditional methods would have taken decades, but by using assembly-line robots and new protocols, they expect to finish the human brain atlas in four years. Here's how it's done.



1) As soon as the institute receives a fresh human brain—fewer than 15 specimens will ultimately be used to create the atlas—it's immediately hand-sliced into 5-mm slabs, which are frozen.

2) Using a machine called a microtome, technicians shave each slab into thousands of transparent slices only a few microns thick. These are mounted on 2 x 3-inch barcoded glass slides.

3) In a process called in situ hybridization, specialized robots work round the clock using fragments of RNA to probe each sample for a particular gene, which is stained with colored dye.

4) Robotic microscopes equipped with high-speed loaders take digital photographs of each slide. The intensity of dye color is used to quantify the amount of gene expression in the tissue.

5) The complete atlas, correlated by both gene and location, is stored on the institute's servers. Powerful tools to explore the data will be available for free to all researchers at brain-map.org.

There's something ironic about Allen, cofounder of a software empire, funding an exhaustive atlas of our neural hardware. (He established the institute with a donation of $100 million.) For decades, many cognitive scientists insisted that the physical brain was largely irrelevant to the study of the mind. It didn't matter whether the human operating system was running on a real cortex or a set of silicon microchips—the software was everything. Given Allen's background—this was the man who helped develop MS-DOS 1.0, after all—he might have been expected to ally with the software crowd in the belief that the 1s and 0s were more important than the anatomical details. Instead, Allen decided that our operating system could run only on one very particular kind of computer. "There are so many intricacies to our brain that won't be understood unless we start to look at the system as a whole," he says. "All these different details don't operate in isolation. But how do they work together to create such a powerful machine?"

The cavernous and antiseptic main lab on the second floor of the Allen Institute is dominated by five big black boxes, each the size of a Smart Car. These are robots, specially constructed by lab-automation company Tecan. At the center of each is a glass window, through which all the action can be observed: A metal arm equipped with a series of long plastic pipettes moves endlessly back and forth, squirting a variety of liquids onto slices of brain. The accompanying mechanical noises—a comforting chorus of squeaks, clanks, and beeps—sound like the androids from WALL-E. At the moment, each robot is processing 192 brain slices per day, allowing the lab to analyze nearly a thousand every 24 hours. (Other bots perform more specialized tasks, like delicately adding glass covers to the tissue samples.) They work through the night, continuing to do science while their human counterparts sleep.

Before a single brain was dissected, back when the atlas was still purely hypothetical, Allan Jones realized that the most difficult challenges wouldn't be scientific. All the necessary tools were available, and there were no theoretical obstacles. Instead, Jones worried about the seemingly infinite amount of data required. "There really was no model for this type of project," he says. "There was no earlier map that we were trying to improve or update. And the reason there wasn't another map was because it didn't seem possible."

What the institute needed was someone who could translate its epic ambition into an efficient production process, in which thousands of brain slices would be collected and assessed *every day*. This led Jones to hire Paul Wohnoutka, a former Boeing engineer with decades of experience managing complex manufacturing systems. ("I thought a commercial airliner was the most challenging thing I'd help build," he says. "I was wrong.") Wohnoutka has an earnest Midwestern demeanor; his speech quickens with excitement when he starts describing the details of his assembly line, like the colored barcodes used to classify microscope slides. His first priority was to standardize everything so that each slice was put through the exact same process, which he detailed in thick binders filled with instructions. "Scientists are used to working by themselves, so they can get pretty suspicious when you start talking about industrialization," Wohnoutka says. "But all we're really doing here is applying some basic principles that manufacturing companies learned decades ago. It only seems strange because we're making science, not widgets."

In biology, most experiments are done in small batches by postdocs and grad students. That would never work here. Just consider the technical difficulty of mapping the entire brain: Each organ must be cut into thin slices that are measured in microns. These slices—several thousand per brain—are then immersed in a concentrated RNA solution to probe for a specific gene. The basic idea is that the RNA will bind to its complement in the brain cells. (This is made possible by the interwoven nature of the double helix, with one strand automatically attaching to the other.) The tissue is then washed with a series of antibodies and chemicals that attach to the RNA, causing the molecule to become visible. In the Allen Brain Atlas protocol, the cells containing the RNA are stained a washed-out violet, the color of spilled wine, with higher levels of gene expression leading to darker shades. This experimental method is known as in situ hybridization, and it has been a staple of bench science for nearly 40 years. But doing it on this scale is utterly without precedent, possible only because the institute perfected its high-throughput protocol.

There are no robots on the first floor of the Allen Institute. Instead, it's dominated by a surprisingly antiquated piece of furniture. It looks, at first glance, like the card catalog for a vast library—large cabinets with hundreds of small, meticulously labeled drawers. Opening one triggers a clattering of glass, the shifting of microscope slides. Each slide is blank except for what appears to be a greasy fingerprint in the center. Not until it's held up to the light does the content become clear: The smudge is actually a sagittal slice of mouse brain.

These slides—there are more than 250,000—provided the raw data for the mouse brain atlas, the first neural map constructed by the institute. While

the mouse atlas is sometimes described as a mere precursor to the human version—a way to perfect the protocols and show that the robots were ready—it's actually been an invaluable resource for gaining insight into the human brain. After all, natural selection is an inveterate tinkerer, and every animal brain is made out of the same basic shopping list of used biological parts. "It might be disconcerting for some people to think about how much our brain has in common with the brains of rodents," Jones says, "but that's just how it is."

Traditional methods would have taken decades, but by using assembly-line robots and new protocols, the Allen Institute expects to finish the human brain atlas in four years.
Photo: David Clugston

The mice were exquisitely standardized: Only 56-day-old males of the C57BL/6J strain were used. To keep track of all the samples, the glass slides were labeled with unique barcodes identifying where in the brain they came from and which genes they were being tested for. When scientists want to check a specific slide, they simply whip out a handheld barcode reader and all the relevant information instantly appears on a computer screen. If it weren't for this data-management system, designed by Wohnoutka, the institute would be utterly overwhelmed by its own experimental results. "The barcodes are just our version of the lab notebook," Wohnoutka says. "When you have a million-plus samples, you simply can't write stuff out by hand."
Once the *in situ* hybridization protocols were tweaked for the Tecan robots, the gene mapping was relatively straightforward. The mouse atlas project soon became a matter of efficient repetition, as the factory floor churned out more than 1,000 slices of mouse brain every 24 hours.
But the flood of data exceeded the ability of scientists to analyze it. Glass slides started to gather in neglected piles; there were too many mouse brains and not enough microscopes to study them. "We quickly realized that you can't industrialize just one part of the system," Jones says. "You have to industrialize everything, or else you'll be stuck with all this information that you can't understand."
So the next challenge was finding a way to digitally photograph every slide. Given the output of the lab, it was obvious that robotic microscopes would be required. Unfortunately, no such technology existed, which meant that the institute had to build its own. The researchers rigged 10 Leica 600B microscopes with glass-slide loaders, barcode readers, and small computers running image-analysis software. The machines are mesmerizing to watch—the lenses constantly zoom in and out like metal eyes. Every two seconds, a new snapshot of a stained brain slice enters the atlas. To date, these microscopes have taken more than 85 million photographs.
The data then travels downstairs to the massive computer room, where rows of hard drives and CPUs are stacked in metal racks connected by thick tangles of black wires, like nerve fibers. Two 20-ton air conditioners make the space sound like a wind tunnel. (When the AC briefly failed last year, the room went from 68 to 92 degrees Fahrenheit in less than 20 minutes.) Once an image enters the cluster, an algorithm quantifies billions of individual neurons and translates them into a statistical "heat map" of gene expression. This is the heart of the project, the part that turns the data into something that actually looks like an atlas.
Michael Hawrylycz, director of informatics at the institute, helped design the software. Although colleagues often tease Hawrylycz for being absentminded and messy—the day I met him, he was wearing his T-shirt inside out and his office was a labyrinth of piles—his innovations have allowed the atlas project to classify and categorize the astonishing amount of data. "I make sure scientists can find what they're looking for," he said, before trying in vain to find a scientific paper that was lost somewhere on his desk.
At first, Hawrylycz and others assumed that the most common search would be anatomical—in other words, that scientists would use the atlas to see

which genes were expressed in a particular brain area, like the hippocampus. However, the unexpected complexity of the brain meant that such broad searches returned way too much information; the old boundaries were suddenly useless.

This led Hawrylycz and his team to invent a new set of search tools. First, they divided the mouse brain into 53,000 voxels, or microscopic cubes. This enabled scientists to quickly figure out the most important genes in that bit of brain, since they could see which were most highly expressed. They could also compare the gene expression patterns of various voxels to one another. Do you want to know what other brain area most resembles a particular circuit of layer-5 neurons in the left cerebral cortex? Just click on the circuit and a colorful map is superimposed on the mouse brain. The dark red areas represent voxels that are similar, while navy blue signifies an area expressing a very different set of genes. (Imagine if Google Maps let you compare any street in Seattle with every other street in every other city in the world for thousands of variables and you can begin to understand the power of such a tool.) "We call it an 'unbiased' spatial search, because it allows you to look past these old anatomical maps and pull out all sorts of unexpected correlations," Hawrylycz says. "The goal is to let people make their own maps."

This means that once the human atlas is complete, a scientist studying autism or Alzheimer's or human intelligence will be able to quickly generate a snapshot of the brain that reflects the specific genes they're interested in.

In January 2007, after four years of high-throughput experiments and painstaking programming (and a cost of $45 million), the institute published a *Nature* paper describing the methods and results of the mouse atlas. (When the project was completed on time and under budget, the British medical journal *The Lancet* compared Allen's venture with his former Microsoft partner's plan to cure malaria: The headline read "PAUL ALLEN 1, BILL GATES 0.") The entire database was made available for free online at brain-map.org.

"The atlas has become an essential tool for the field very quickly," says Susumu Tonegawa, a Nobel laureate and professor of neuroscience at MIT. He relies on the maps when creating "knockout" strains of mice—rodents that are missing a specific set of neural genes. "These are animals that at first appear normal," Tonegawa says, "but when you look closer you notice that they have deficits in learning and memory depending on what you have interfered with." By determining where each of these deleted genes is expressed in the mouse atlas, Tonegawa can quickly identify the circuit of cells he erased, which shows him exactly which parts of the brain were affected by the genetic mutation. "I can see what is broken," he says, "and that lets me understand how it works."

One unexpected—even disheartening—aspect of the Allen Institute's effort is that although its scientists have barely begun their work, early data sets have already demonstrated that the flesh in our head is far more complicated than anyone previously imagined.

The brain might look homogenous to the naked eye, but it's actually filled with an array of cell types, each of which expresses a distinct set of genes depending on its precise location. Consider the neocortex, the so-called CPU of the brain: Scientists assumed for decades that most cortical circuits were essentially the same—the brain was supposed to rely on a standard set of microchips, like a typical supercomputer. But the atlas has revealed a startling genetic diversity; different slabs of cortex are defined by entirely different sets of genes. The supercomputer analogy needs to be permanently retired.

Or look at the hippocampus, the crescent-shaped center of long-term memory. Until recently, this small fold of tissue in the middle of the brain was depicted as neatly divided into four distinct areas. But data from the atlas has rendered the old maps not only obsolete but flat-out misleading. Even a single hippocampal area can actually be subdivided into at least nine discrete regions, each with its own genetic makeup.

Scientists at the institute are just starting to grapple with the seemingly infinite regress of the brain, in which every new level of detail reveals yet another level. "You can't help but be intimidated by the complexity of it all," Jones says. "Just when you think you're getting a handle on it, you realize that you haven't even scratched the surface." This is the bleak part of working at the Allen Institute: What you mostly discover is that the mind remains an immense mystery. We don't even know what we don't know.

But Jones and others aren't ready to surrender. They remain excited by the idea of working on the frontier of science, by the possibility that their maps will allow others to make sense of this still inscrutable landscape. In other words, they are waiting for the future, for some scientist to invent an elegant theory that explains their enigmatic data. Jones likes to compare the current state of neuroscience to 19th-century chemistry. At the time, chemists were strict empiricists; they set substances on fire and then recorded the colors visible in the flames. Different chemicals produced different spectrums of light, but nobody could make sense of the spectrums. The data seemed completely random. But then, with the discovery of quantum mechanics, scientists were finally able to explain the colored light—the unique rainbows were actually side effects of subatomic structure. Such is the faith of scientists: Nature must always make sense.

The five specially constructed robots at the allen institute can each process 192 brain slices a day.
Photo: David Clugston

But what if neuroscience isn't like chemistry? The brain, after all, is a byproduct of evolution, an accumulation of genetic accidents. The data that looks so arbitrary might actually *be* arbitrary. If that's the case, having a precise atlas of the brain won't lead to a unified theory—because such a thing can't exist.

Occasionally this doubt seeps into conversations about the atlas, as the scientists wonder aloud whether these 3 pounds of tissue can ever be understood. "The brain is just details on top of details on top of details," Hawrylycz says. "You sometimes find yourself asking questions that don't have answers, like 'Do we really need so many different combinatorial patterns of genes?' Well, it doesn't matter if we *need* to be this way. It's the way we are. The brain doesn't care about making our job easy."

There are also several unresolved technical problems. For example, the human brain is 2,000 times larger than the mouse brain, which means that even the industrialized protocols of the Allen Institute can't generate *all* the necessary amounts of data. The scientists are forced to augment the refined maps of in situ hybridization with cruder techniques, which provide a measurement of gene expression in particular brain areas but not at the cellular level.

"The problem with this data," one researcher told me, "is that it's like grinding up the paint on a Monet canvas and then thinking you understand the painting." The scientists are stuck in a paradox: When they zoom in and map the brain at a cellular level, they struggle to make sense of what they see. But when they zoom out, they lose the necessary resolution. "We're still trying to find that sweet spot," Jones says. "What's the most useful way to describe the details of the brain? That's what we need to figure out."

And then there are the theoretical questions. Although the scientists are determined to create a universal map of the brain—a generic guide to its gene expression—such an abstraction doesn't actually exist. There is no single human brain, just as there is no single human genome. As a result, the scientists must determine what sort of brains should be included in the atlas. (These issues are especially important given the limited supply of available human specimens. While thousands of nearly identical mice were used to create the mouse atlas, its human counterpart will be based on fewer than 15 highly distinct individuals.) When I was at the institute, the scientists were struggling to define what it meant to be "normal." Is it normal to smoke cigarettes? Is it normal *not* to drink alcohol? What about a cortex of someone who has taken antidepressants? Or spent years in psychoanalysis? Or committed a violent felony? Is anybody normal? How do you standardize the individual?

Although the human atlas is years from completion, a theme is beginning to emerge: Every brain is profoundly unique, a landscape of cells that has never existed before and never will again. The same gene that will be highly expressed in some subjects will be completely absent in others. Important drug targets, like serotonin receptors, will exist in a disparate set of brain areas depending on the individual. This variation is even visible at a gross anatomical level—different people have differently shaped cortices, with different boundaries between anatomical regions. (This is why, for instance, neurosurgeons have to painstakingly probe the cortex during surgery.) If the human atlas is like Google Maps, then every mind is its own city.
"It can seem like there's an infinite number of variables to consider when you look at the human brain," says Elaine Shen, a manager at the institute. "We're making a genetic map, but what if the map isn't detailed enough? Or what if each brain is so different in expression patterns that we can't make sense of it?" She and her colleagues are convinced, however, that the only way to solve these unknowns is to look at the data, to break the brain apart and try to measure everything. "Once all the data is out there, someone else is going to connect the dots," Jones says. "All we want to do is make that scientific leap possible."
Jonah Lehrer(jonah.lehrer@gmail.com) *is the author of* How We Decide and Proust Was a Neuroscientist.

See Also:
Memory Switch Could Enable Brain Hacks
Brain Scanners Know Where You've Been
Dial H for Happiness: How Neuroengineering May Change Your Brain
Rewiring the Brain: Inside the New Science of Neuroengineering