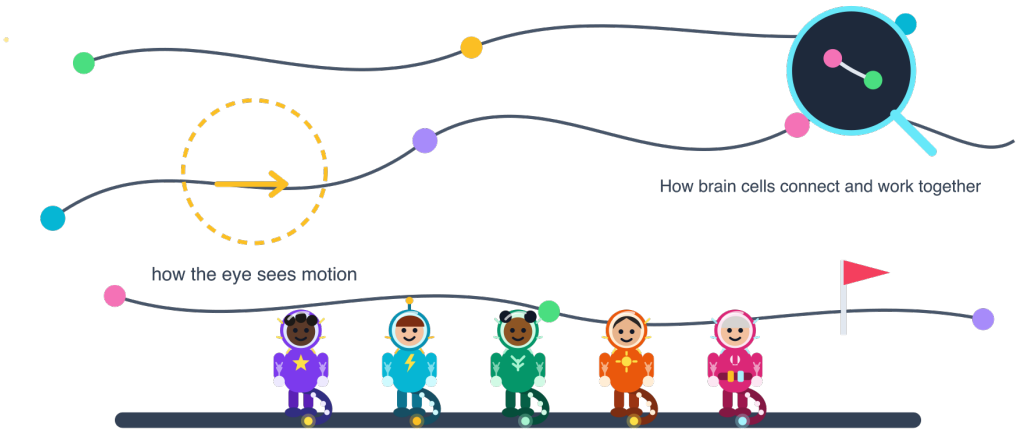
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The Neuronauts and the Mystery of the Brain Map

How do we map a brain—and know our map is right?



Will Gray-Roncal and Sydney Floryanzia

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The Neuronauts

and the Mystery of the Brain Map



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About this adventure

The Neuronauts are imaginary explorers, and their particular mystery is invented. The tools and scientific questions in their adventure are real.

The guide at the back shows where the story meets the science.

VERSION 1 — PROOF COPY
By Will Gray-Roncal and Sydney Floryanzia

This book was built with our expertise in scientific communication, mentoring, and connectomics, with the help of artificial intelligence tools. All results were carefully reviewed, and the authors take responsibility for the content. Please let us know if you see any errors or omissions.

4 November 2026

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Words for the expedition

Neuron. A nerve cell that receives and sends signals.

Axon. A cell extension that carries signals toward other cells.

Dendrite. A branch that receives many of a neuron's inputs.

Synapse. A junction where one cell passes a signal to another.

Glia. Cells that help maintain and support the nervous system.

Circuit. Connected cells that can work together.

Hippocampus. A brain region important for memory.

Connectomics. Studying and mapping neural connections.

Connectome. A map of connections in a nervous system or region.

AI. Computer methods trained or designed to perform tasks.

Electron. A tiny particle used to make electron-microscope images.

Motif. A small wiring pattern; its shape alone does not reveal its job.

Reconstruction. A tracing or model built from pictures.

Alignment. Lining pictures up to follow a structure.

Segmentation. Labeling which picture regions belong together.

Proofreading. Checking a reconstruction and correcting errors.

Uncertainty. What the evidence does not yet let us tell.

Hypothesis. An explanation or idea we can test.

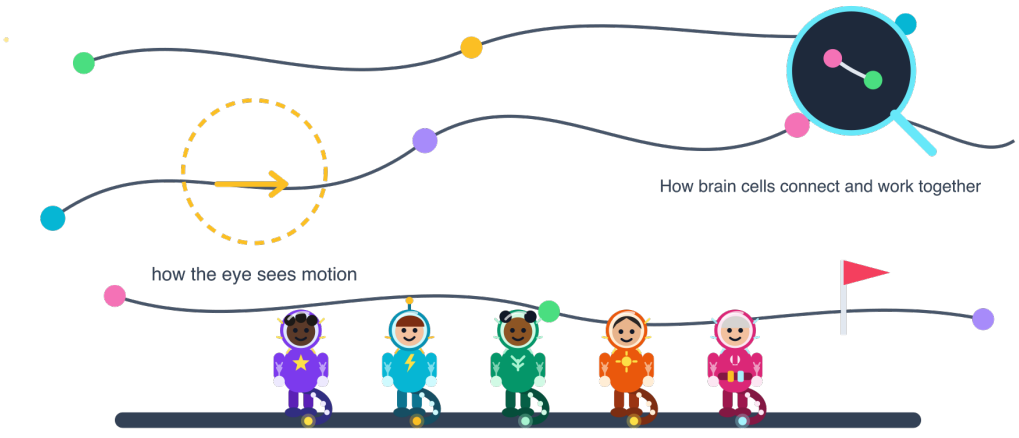
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How do we map a brain—and know our map is right?



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Why take this journey?

Remembering someone you love. Finding your way home. Telling a friend what you need. Brain cells working together help make these moments possible.

When brain circuits are disrupted, everyday life can become harder—for a person and the people who care about them.

Connectomics studies how nerve cells connect. We build maps to understand how these circuits work, what can go wrong, and what might help.

That is why this expedition matters. Behind every tiny connection is a much bigger question about our lives.



A research path, with experiments and clinical validation between steps.

Health context: NIH BRAIN Initiative; research links at the back.

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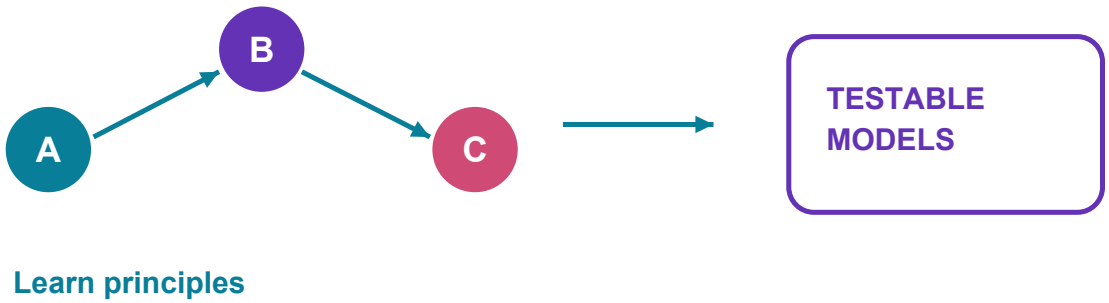
Small power, remarkable abilities

Your brain helps you move, learn, and adapt using about 20 watts of power from food. [12]

An adult brain is about 2% of body weight, yet uses about 20% of the body's energy at rest. Keeping its cells working takes energy! [25]

Could its circuits teach us to build computers that use less energy and handle unfamiliar situations? Scientists connect brain research with artificial intelligence, or AI, in a field called NeuroAI. [14]

A fair comparison asks brains and computers to do the same task and counts the energy each uses.



Learn principles

Research direction, not a claim that a brain wins every benchmark.

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When connections matter

Energy powers the signals that brain cells pass to one another. Illness can change those connections or the way signals travel. Scientists sometimes call disorders involving changed connections connectopathies.

How do circuits contribute to seizures in epilepsy? How does damage disrupt communication in dementia?

Not every difference means illness. Brains also differ as we grow and learn.

Maps can help us investigate these questions. To turn a finding into a treatment, we must test how it works and whether it safely helps people.



A research path, with experiments and clinical validation between steps.

Read alongside references [10], [11]. A research map is not a diagnosis.

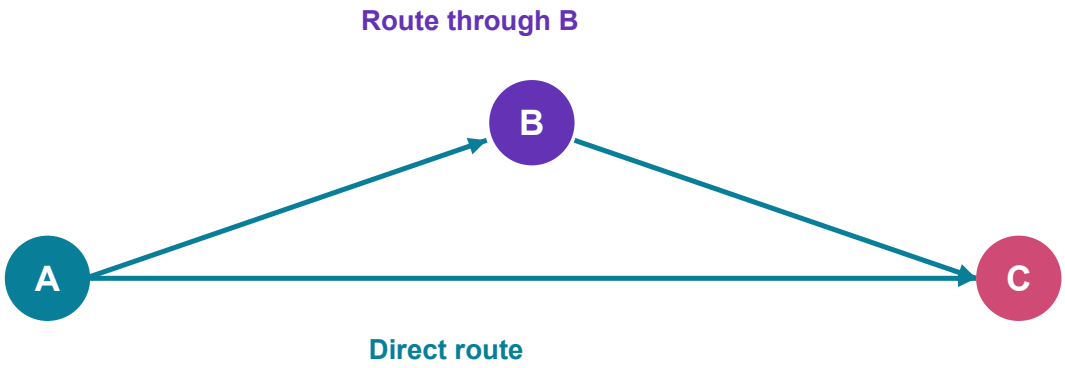
The grand challenge

Scientists have mapped an adult fly brain and detailed pieces of mouse brains. [5], [6]

A human brain is far larger than a fly brain. To explore human illness at this level of detail, we need to develop and test tools for much bigger, trustworthy maps.

Then we can compare maps, uncover clues, and test explanations—working toward benefits for health and computing.

In this story, our imaginary Neuronauts crew explores a mouse hippocampus, a brain region important for memory. The final discovery is invented, but the scientific questions are real.



Mission framing: NeuroTrailblazers, Why Map the Brain; references [5], [6], [13].

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Are we there yet?

This is an exciting time to explore brain maps: several major milestones are recent. Here is what some of them made possible.

1986

Worm nervous system

White and colleagues mapped its wiring. [16]

2023

Larval fly brain

Winding and colleagues traced a whole insect brain. [17]

2024

Adult fly brain

FlyWire reconstructed 139,255 neurons and their links. [5]

2025

Mouse visual cortex

MICrONS linked activity measurements with wiring. [6]

NOW

Develop and prove the technology

Build bigger maps to search for answers.

NEXT

Compare maps and test explanations

Investigate what circuits do and why they differ.

THEN

Scale, validate, and translate

Work toward benefits for health and computing.

Selected milestones. You can join the journey: page 43. Future benefits require further tests.

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Why look this closely?

Are two tiny branches connected, or just close together? To tell, we must see where they meet.

An electron microscope uses a beam of tiny particles called electrons to make pictures of preserved tissue. These pictures can show cell boundaries and the structures of a synapse—a junction where one cell sends a signal to another.

We still need people and computers to trace the cells and check the map. Other tools help answer other parts of the puzzle:

MRI	Uses magnets and radio waves to look inside a living brain. It shows large structures and clues to pathways, not single synapses.
Light and cell labels	Microscopes and labels help us find particular cells. Some labels grow brighter or dimmer as cell activity changes.
Electrical recording and stimulation	Record electrical signals. Then deliberately change activity and measure what happens to test an idea, or hypothesis, about the circuit's job.
Computer models	Build a simplified circuit in a computer. Predict what it might do, then compare with real measurements.

Complementary evidence: references [1]–[3], [6], [18], [19].

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Meet the crew



Captain Cortex brings the team's observations together and helps them decide what to investigate next.



Axon scouts ahead and sends news of each discovery back to the team. Sometimes a little too quickly!



Dendra is a good listener. She gathers clues from the others and keeps careful notes.



Syn makes connections—between teammates, between clues, and between one question and the next.



Glia supports the whole team, caring for their tools and helping everyone work through trouble.

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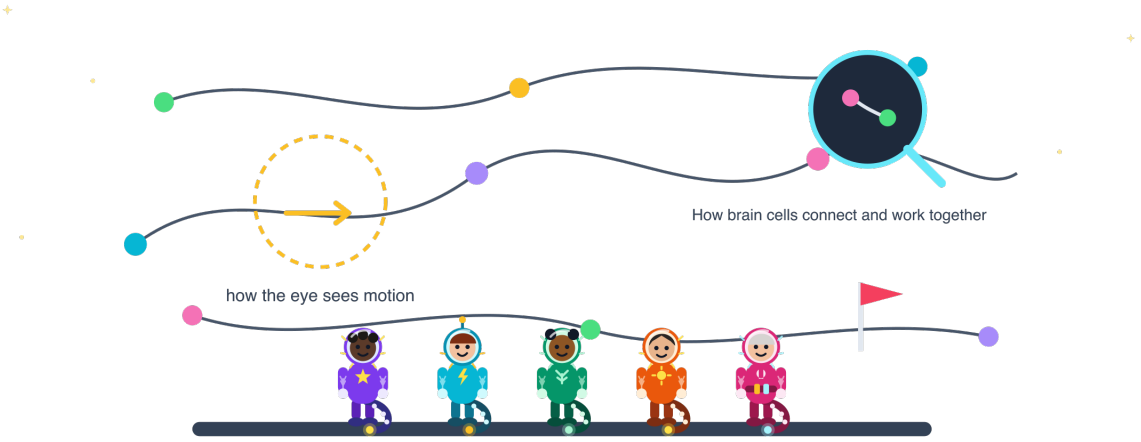
Your place on the team

Explorers crossing oceans or traveling into space need instruments they can trust. Our crew does too.

Microscopes reveal tiny details. Computers help build maps. Careful checks tell us what we can believe.

These tools and steps make the mission possible. Without them, we could mistake an error for a discovery.

Keep asking: What information or tools would help us figure this out?



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A curious wiring pattern

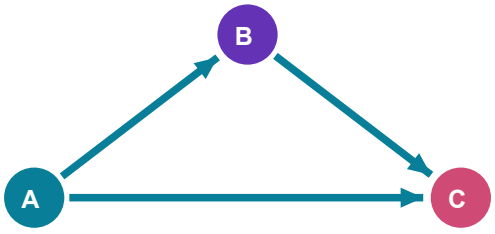
The Neuronauts stumbled upon a map of a mouse hippocampus—a brain region important for memory.

They had seen the same small wiring pattern on similar maps before: A reached C directly, and through B.

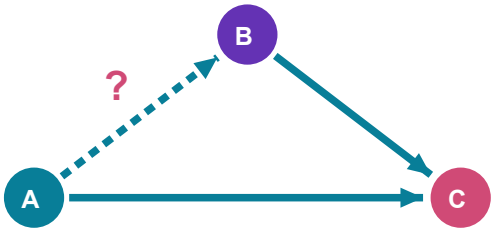
But here, one route seemed to stop.

“Oh, that’s interesting!” said Axon. “Why is this one different on this map?”

Seen on earlier maps



This example: check the evidence



Arrows show connection direction. A dashed arrow marks an uncertain connection.

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Three possibilities

Axon sent a message to the team.

“One route seems to stop!”

Dendra listened. “You said seems. Let’s keep that uncertainty in our notes.”

Captain Cortex listed three possibilities: a real ending, a missing picture, or a tracing mistake.

Glia brought up the original images. “Let’s give everyone the evidence they need.”



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A map of what?

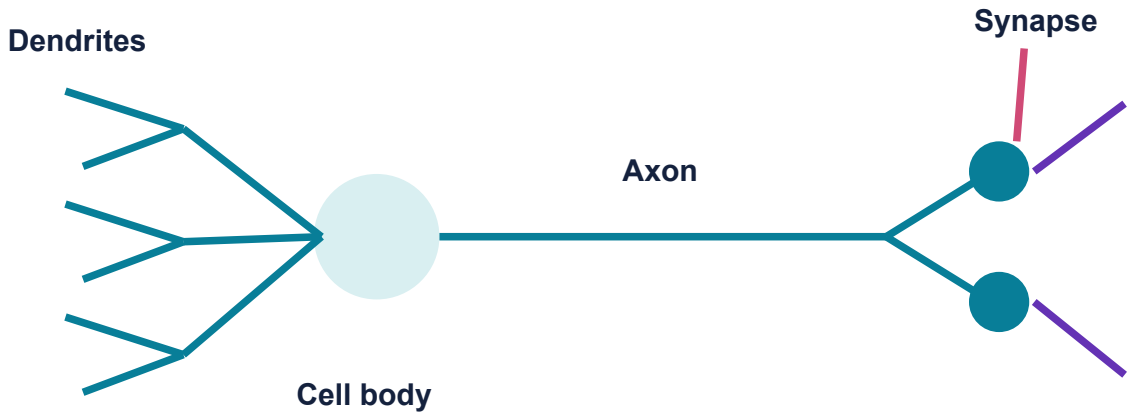
Their map began with a tiny piece of brain.

Inside it, nerve cells called neurons sent out long extensions and branching arms.

Some met at special junctions called synapses.

Syn brightened.

“Those are my favorite places.”



Simplified neuron and junctions; not to scale.

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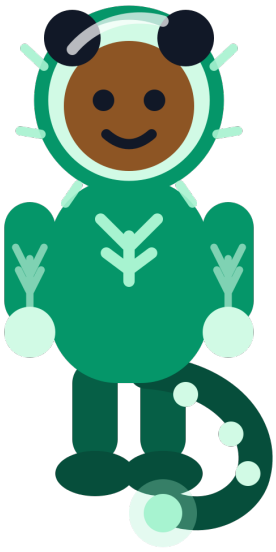
Clues from earlier explorers

“Has anyone seen this before?” asked Syn.

Dendra listened as the crew compared ideas, then opened earlier hippocampus studies.

Syn connected the clues. “Those researchers studied direct and indirect routes too. Their experiments might help us choose what to test.”

“First, we must check our wiring,” said Captain Cortex.



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Keeping the clues

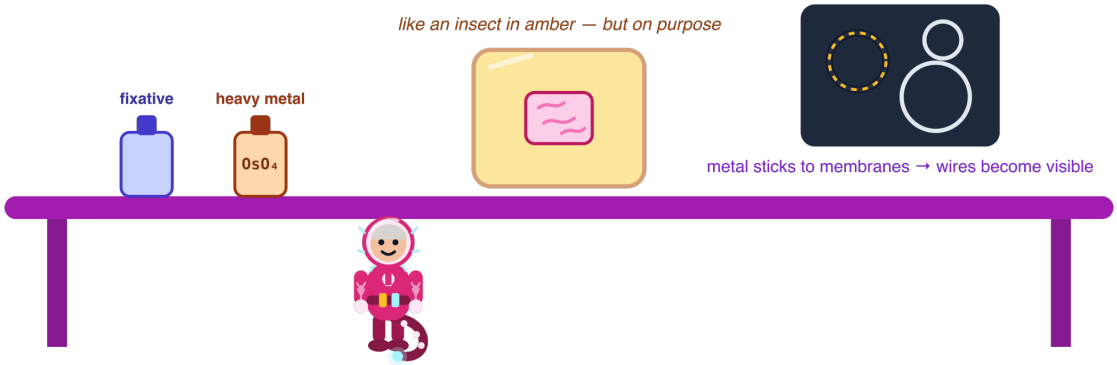
To understand their map, the crew followed its trail backward.

They started with real brain tissue.

Glia explained how scientists preserve its tiny structures.

“An ocean explorer needs a vessel that keeps water out. We need preparation that keeps the details we want to study.”

The mission began before the first picture.



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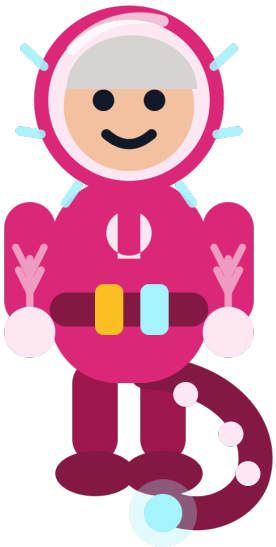
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Check from the beginning

Special stains marked cell boundaries and helped them stand out in the microscope. A firm material supported the tissue for cutting.

“Can the way we prepare the tissue change what we observe later?” asked Dendra.

“It can,” said Glia. “That is why we check our work from the beginning.”



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Thin enough to see

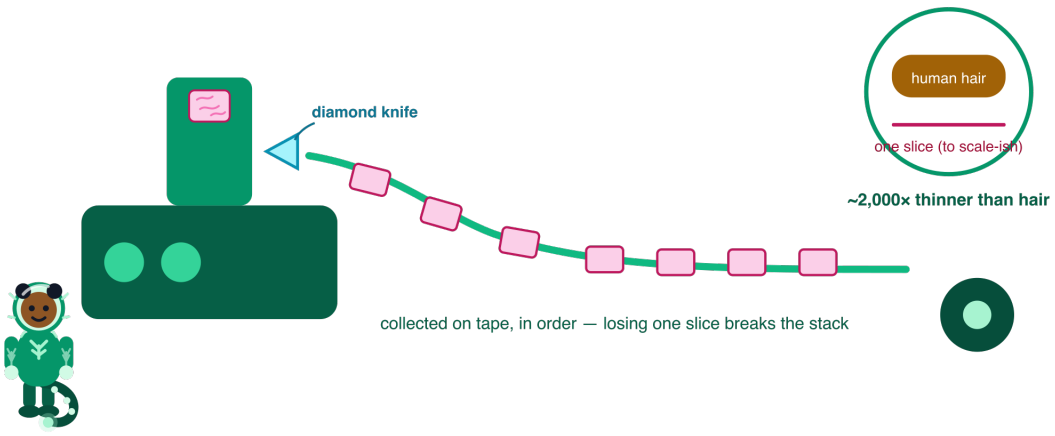
Next, Dendra watched a machine called an ultramicrotome cut an extraordinarily thin slice.

Then another.

Then another.

One slice showed only a little of each winding branch. In order, the slices could reveal its path through the sampled tissue.

“We’ll need the whole sequence,” Dendra said.



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Keep the sequence

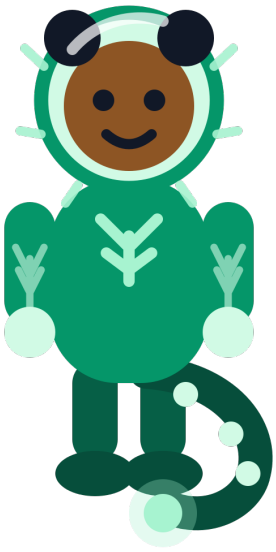
Axon peered at the growing collection.

“What if we mix them up?”

“What if one tears?” added Syn.

Dendra checked the labels.

“Then we need to know. A missing piece of evidence belongs in the notes.”



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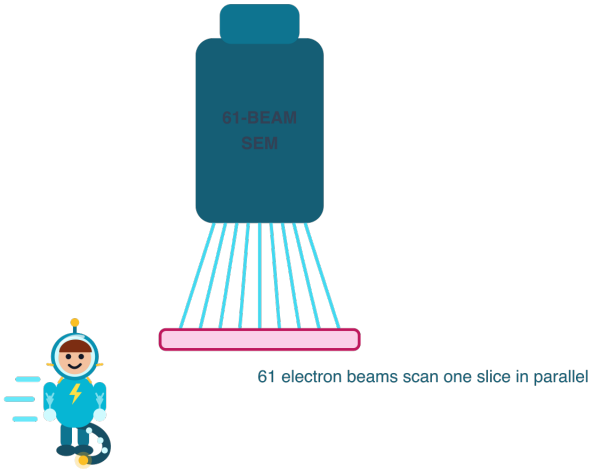
Pictures from electrons

At the electron microscope, Axon finally found a machine that appreciated speed.

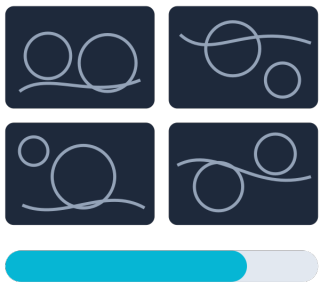
It used electrons to reveal details far too small for their eyes.

The screen filled with shapes in shades of gray.

“So many clues!”



61 electron beams scan one slice in parallel



data collected: ~2,000,000 GB per mm³

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Enough clues?

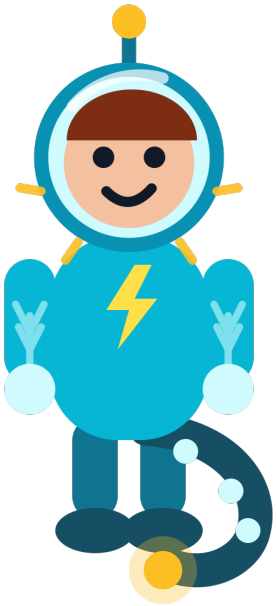
Axon sent back a warning. “This patch is blurry.”

Dendra listened and marked the location.

Glia helped check the instrument and take another picture of the slice.

“Like checking a telescope before trusting a distant light,” said Syn.

A fast picture helped only if they could read it.



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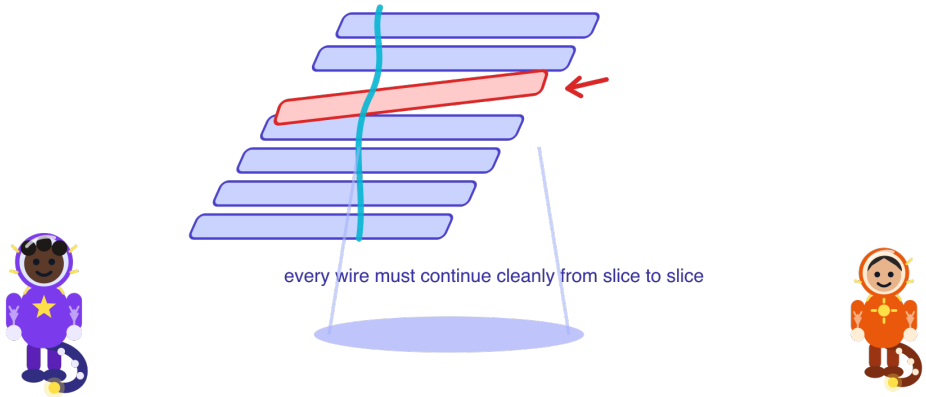
Putting pictures together

The pictures arrived like pages from a very unusual book.

Some were shifted. Some were tilted.

Dendra searched for details that appeared in neighboring pictures.

“Here. And here. These belong together.”



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Follow the evidence

With help from the computer, the crew lined up the pictures.

The wandering branch became easier to follow.

But Captain Cortex stopped Axon from smoothing away an awkward bend.

“We must follow the evidence—even when it makes a messy picture.”



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The computer’s first answer

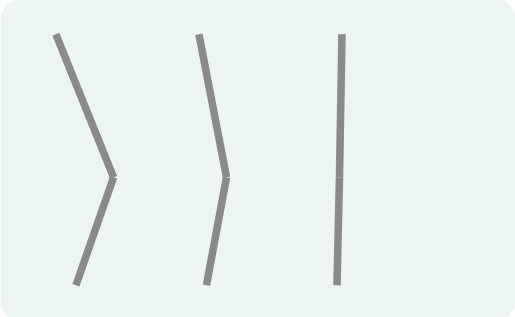
Now an AI program helped trace the cells across the pictures. It had learned from examples how to recognize their boundaries.

One branch became blue. Another orange. Another green.

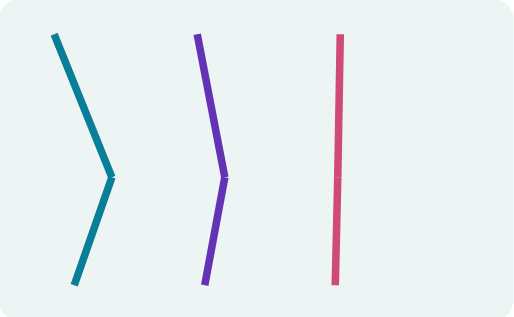
“The colors are labels,” said Glia. “They help us follow each cell. But the computer can make mistakes.”

Syn leaned closer. “Let’s check.”

Original shapes



Labels to check



Compare each label with the original. Color is an annotation, not the cell’s real color.

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A suspicious join

Then orange spilled into blue.

Two neighboring branches had become one labeled shape.

“Our two routes!” cried Axon.

“Or a labeling error,” said Syn.

A false join could invent a route that no neuron actually made.

How could they check? They would revisit the original pictures and learn from carefully reviewed examples.



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Learning to look

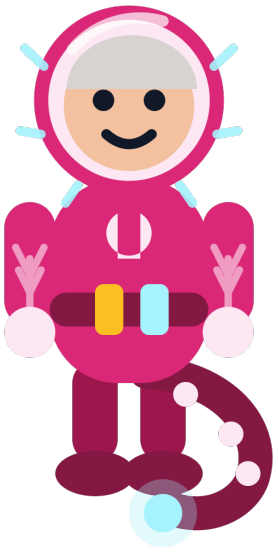
Glia set up a practice case and made room for everyone to try.

Each Neuronaut marked a boundary. Their marks did not agree.

Dendra listened to each explanation before adding her own.

Syn noticed the connection: “We’re all uncertain at the same blurry edge.”

Now they knew where to look together.



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Practice with feedback

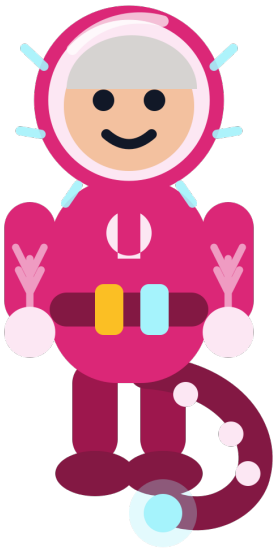
They compared their marks with a carefully reviewed example.

Glia pointed out a boundary Axon had missed.

Axon tried again, this time explaining each step.

The next tricky case looked less mysterious.

And when the picture was unclear, the crew learned to ask for another review.



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Back to the gap

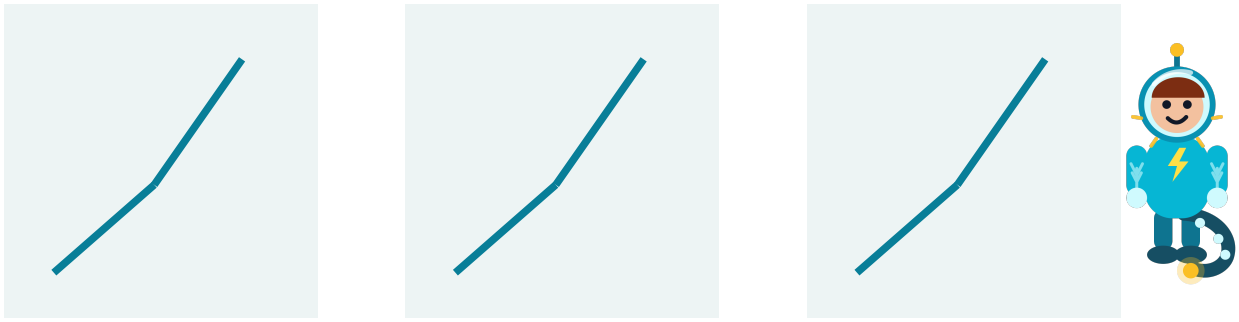
Axon reported the gap in the possible route from A to B.

“I think we can bridge it.”

Dendra listened. “What did you see that supports that?”

Glia placed neighboring pictures side by side.

Together, they followed the branch through one slice, then the next.



Slice 1 **Slice 2** **Slice 3**

Follow the same branch through neighboring pictures.

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One mystery solved

“There!” Axon sent the discovery back to the crew.

“The branch continues, but its color label stops.”

Syn connected the clues. “Then the gap is in the tracing—not the cell.”

Glia helped check the evidence again before joining the labels.

Their tools and teamwork had solved one mystery.



GO DEEPER / PAGE 50

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Another gap

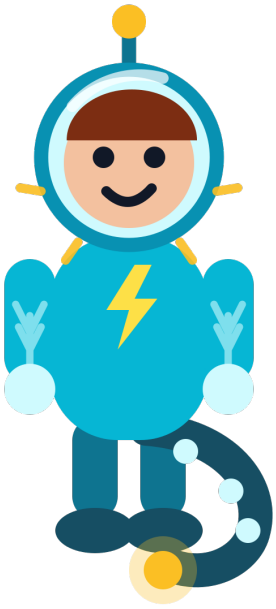
Farther along, they found another gap.

This time a damaged section hid the evidence.

Axon looked from one side to the other.

“It probably continues.”

“Possibly,” said Captain Cortex. “But we cannot tell from these pictures.”



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The question mark stays

Dendra marked the place:

Uncertain. More evidence needed.

“Do we leave it like that?” asked Syn.

“Yes,” said Glia. “And make sure the next explorer can see why.”

The map kept its question mark.



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Close is not connected

Around the next bend, two branches passed very close together.

“A connection!” cried Syn.

Captain Cortex raised a hand.

“What would help us know?”

Syn opened the original images.



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Looking for a junction

At one meeting place, the crew found the tiny structures used to recognize a synapse.

At another, they did not find enough evidence.

Syn marked the first.

“Being neighbors doesn’t tell us the whole story.”



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The pattern emerges

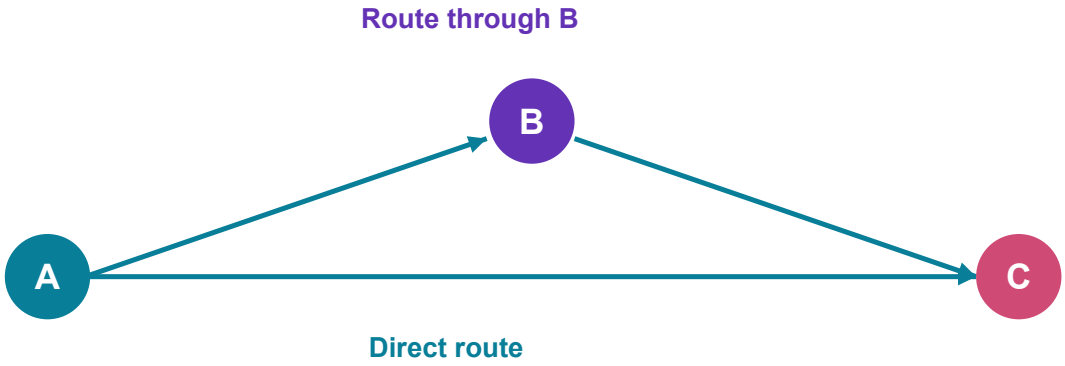
The images supported three connections: A to B, B to C, and A to C.

Their two-route pattern was real in this reconstructed example.

Another damaged region stayed uncertain.

“One pattern found,” said Syn.

“Now we can ask what it does.”



Schematic connections, not cell shapes. Arrows do not specify synaptic sign.

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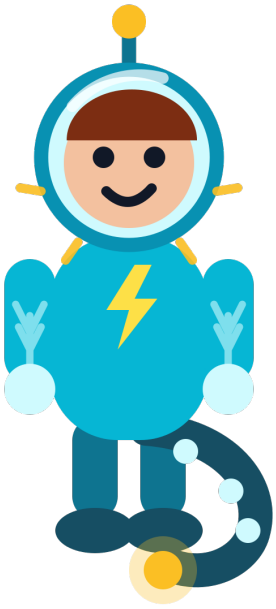
More than a pattern

“Scientists sometimes call a small wiring pattern a motif,” said Captain Cortex.

“But real circuits are more than three circles.”

Different kinds of cells behave differently. A connection can make another cell more or less likely to send a signal. Its strength, timing, and location matter too.

Earlier experiments offered clues. To find out what this pattern did, the crew would need to measure signals and test their ideas.



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Interesting does not mean diseased

Another example still looked different.

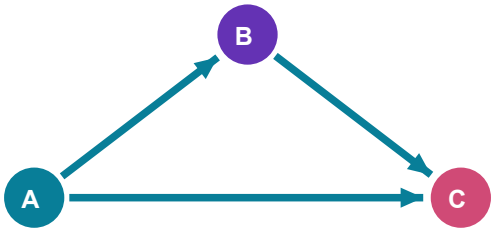
“Let’s look at what other scientists found,” said Dendra.

Had they studied the same kinds of cells, in the same brain region, at a similar age? Were their pictures clear enough to compare?

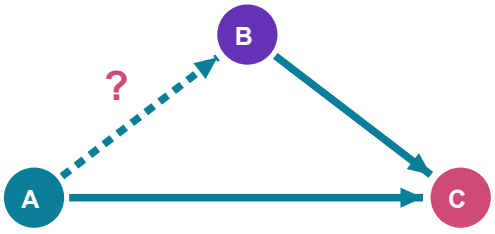
A difference might come from growing, learning, ordinary variation, or a mistake in the map.

Calling it a sign of illness would take much more evidence.

Seen on earlier maps



This example: check the evidence



Arrows show connection direction. A dashed arrow marks an uncertain connection.

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A discovery others can check

Dendra listened while each teammate described what they had checked. Then she wrote their report.

“We found this wiring pattern. We do not yet know its job or what a difference means for health.”

Axon shared the pictures and corrections. Syn linked the report to earlier findings. Glia made sure the uncertain places stayed visible.

Another team could now check their work.



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Why one small check matters

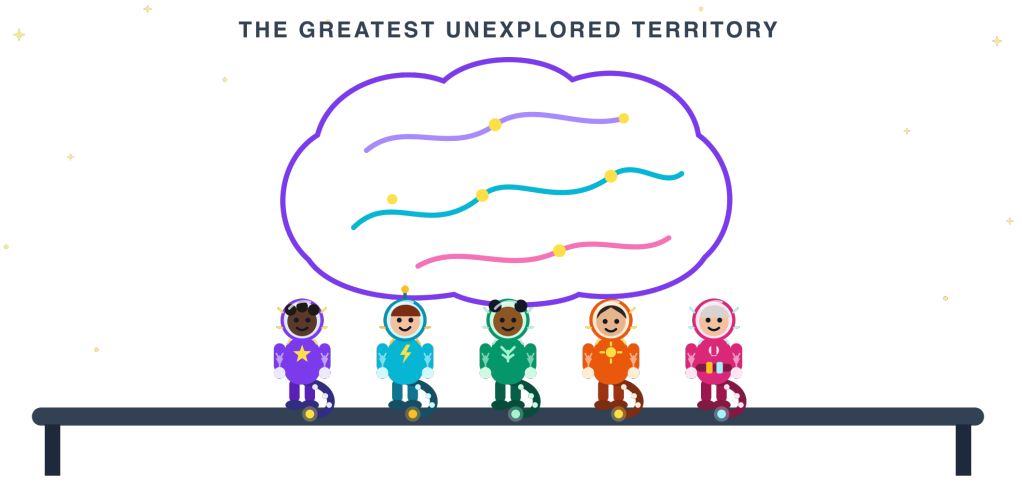
“Why spend so long checking one tiny branch?” asked Axon.

“Because someone else may build their next experiment on our map,” said Dendra.

Syn looked up. “An experiment about memory? Or illness?”

“Perhaps,” said Captain Cortex. “Our map is not a treatment. But trustworthy clues help scientists choose what to test next.”

Small checks can serve a larger purpose.



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Your expedition

Captain Cortex opened the next mission.

Glia checked the tools.
Dendra opened the notebook.
Syn leaned closer.
Axon waited for the question.

There was room at the screen for one more explorer.

What would you investigate?



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THE WHOLE EXPEDITION

Looking back: tissue to images

01

Ask a question

Choose the brain region and pattern to study.

CHECK: Look for clues in earlier research.

02

Keep the tiny details

Preserve the tissue and make boundaries stand out.

CHECK: Note anything the preparation changes.

03

Make the pictures

Cut thin slices and image them with electrons.

CHECK: Check for blur, damage, and missing slices.

04

Line up the pictures

Match details across neighboring slices.

CHECK: Do not bend the evidence to fit an idea.

One serial-section EM workflow. Other acquisition methods differ. References [1]–[3].

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THE WHOLE EXPEDITION

Looking back: images to insight

05

Trace the cells

AI helps label each cell across the pictures.

CHECK: Find false joins and missing pieces.

06

Find the junctions

Look for synapses and identify the cells they link.

CHECK: Close together does not mean connected.

07

Check the pattern

Review the tracing and compare similar examples.

CHECK: Keep track of corrections and uncertainty.

08

Test and share

Measure signals and test ideas about the circuit.

CHECK: Share the evidence so others can check it.

Review can send us back to any earlier step. References [4]–[9], [11], [13].

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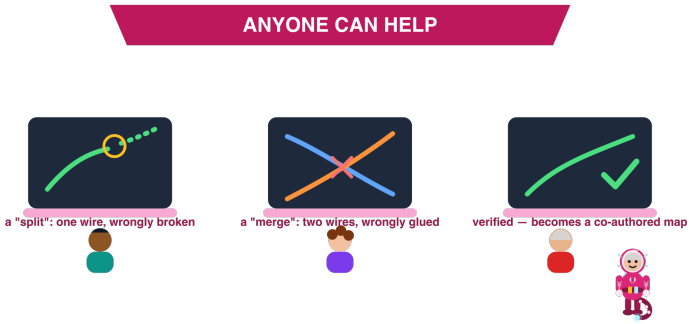
What we can do now

You do not have to be a scientist to care about this work. It asks questions about abilities we use every day—and changes that can affect any family.

You can explore a brain map, ask what a finding really shows, or share an explanation that helps someone else understand.

Want to go further? Practice, feedback, and mentoring can turn curiosity and raw talent into scientific capability: the ability to ask, investigate, judge, and explain.

Checking a map is one place to begin. The goal is learning to do science independently.



Now: develop the tools. Next: compare and test. Then: scale, validate, and translate.

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Where we hope to go

HEALTH FIRST
We hope to understand circuit changes that contribute to illness and use that knowledge to help develop better therapies. Benefits must be tested, not assumed.

SCIENCE AND AI
We hope to learn how brains solve problems and use those ideas to build more adaptable, energy-efficient computers.

First, develop the tools. Next, compare maps and test explanations. Then, expand and validate what works.

The work also gives us something now: shared maps, better questions, and people prepared to pursue them.



Now: develop the tools. Next: compare and test. Then: scale, validate, and translate.

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Go deeper

You have finished the story, but this is just the beginning! Choose a path into the science.

1. GUIDED INVESTIGATION QUESTIONS · pages 46–52
Revisit the story: explore an activity, investigate the evidence, and read the research.

2. TRY A MAPPING INVESTIGATION · page 53
A paper-and-pencil activity for younger readers and partners.

3. READ TOGETHER · page 54
A discussion activity for a class or research group.

4. TECHNICAL COMPANION · pages 55–64
Follow the scientific decisions behind the adventure.

5. CALIBRATING SCIENTIFIC EXPERTISE · page 65
For educators, mentors, and scientists: learning to ask, judge, test, and explain. Proofreading is one example.

RESEARCH AND REFERENCES · pages 66–76
YOUR NEXT QUESTION AND RESOURCES · pages 77–78
REAL HUMAN BRAIN MAPS · pages 79–80

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Guided investigation questions

PAGE 13 · A curious wiring pattern

Explore. Draw A to B, B to C, and A to C. What changes if one route is missing?

Investigate. This teaching diagram is a feedforward loop: one direct path and one path through another cell. A repeated shape alone does not tell us what it does or whether it occurs unusually often.

Read / resources. [4], [13]; technical notes, page 63

PAGE 14 · More than one explanation

Explore. Name a clue that would support each explanation.

Investigate. Ask what could distinguish biological structure, missing images, and a reconstruction error. Some cases remain unresolved.

Read / resources. [2], [4]

PAGE 15 · Cells and junctions

Explore. Find a cell body, a branch, and a junction in a labeled illustration.

Investigate. Axons and dendrites are parts of neurons. A synapse is a specialized junction. The characters are not literal models of cells.

Read / resources. [2], [5]

PAGE 16 · Clues from earlier research

Explore. Find a question an earlier study could help you ask. What would you still need to check?

Investigate. Compare the species, brain region, cell types, methods, and evidence before applying a published explanation to a new example.

Read / resources. [19], [20], page 74

Preparing and collecting evidence

PAGE 17 · Preserving structure

Explore. Describe something that changes before you can examine it closely.

Investigate. Fixation helps preserve tissue structure. It does not preserve a living thought or let this specimen resume normal activity.

Read / resources. [1], [2]

PAGE 18 · Making boundaries visible

Explore. Compare a faint drawing with a higher-contrast copy.

Investigate. Contrast and consistent preparation affect what can be reconstructed. Staining is a laboratory procedure, not a home activity.

Read / resources. [1]

PAGE 19 · Sections become a volume

Explore. Stack different-sized circles drawn on tracing paper. Imagine their three-dimensional shape.

Investigate. Each section samples part of the specimen. Tracing across sections helps reconstruct structures in three dimensions.

Read / resources. [2], [3]

PAGE 20 · Missing information

Explore. Remove one tracing-paper sheet. What information is lost?

Investigate. Track section identity and damage. A plausible interpolation is not an observation of missing tissue.

Read / resources. [3]

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Images and alignment

PAGE 21 · Electron microscopy

Explore. Compare details in a distant photograph and a close-up.

Investigate. Electron microscopy provides fine structural detail. Resolution, contrast, field of view, and acquisition time all matter.

Read / resources. [2], [6]

PAGE 22 · A readable image

Explore. Blur a drawing. Which questions can you still answer?

Investigate. Reimaging can help if the retained section and imaging problem permit it. It cannot restore lost or irreversibly damaged tissue.

Read / resources. [2]

PAGE 23 · Corresponding features

Explore. Shift two transparent copies of a drawing. Find landmarks for alignment.

Investigate. Nearby sections are not identical copies. Real structures change along their length.

Read / resources. [3]

PAGE 24 · A faithful reconstruction

Explore. Could stretching make two different drawings match? Would that be honest?

Investigate. Registration balances correspondence with control of artificial deformation. Visual smoothness alone does not validate a result.

Read / resources. [3]

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Computer assistance and learning

PAGE 25 · Segmentation

Explore. Color connected regions in a black-and-white drawing.

Investigate. Segmentation assigns labels to image regions. These are annotation colors, not the natural colors of cells.

Read / resources. [4]

PAGE 26 · A false merge

Explore. Let one color cross a boundary. How does that change the shapes you count?

Investigate. A merge joins distinct objects in a reconstruction and can alter inferred connectivity.

Read / resources. [4], [5]

PAGE 27 · Comparing judgments

Explore. Independently trace an ambiguous drawing with a partner. Compare disagreements.

Investigate. Agreement is useful, but people can share an error. Use reviewed evidence and explicit criteria.

Read / resources. [4], [5] for reconstruction review

PAGE 28 · Practice with feedback

Explore. Explain a decision, receive feedback, and try a new example.

Investigate. This proposed activity is not a claim that cited studies tested this story's educational effectiveness.

Read / resources. Proofreading tutorials, page 78

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Repair and uncertainty

PAGE 29 · Returning to the images

Explore. Compare an original drawing with a tracing. Which helps check a suspicious line?

Investigate. The colored reconstruction interprets image data. Review underlying evidence and neighboring sections.

Read / resources. [4], [5]

PAGE 30 · A false split

Explore. Divide one continuous shape into two labels, then correct the labels.

Investigate. A split fragments a single object in the reconstruction. Repairing annotations does not change the original tissue.

Read / resources. [4]

PAGE 31 · Insufficient evidence

Explore. Cover the middle of a line. Draw two different paths that could fit beneath it.

Investigate. Several explanations can fit visible evidence. Confidence cannot recover missing data.

Read / resources. [3], [4] for methodological context

PAGE 32 · Recording uncertainty

Explore. Distinguish “unchecked,” “uncertain,” and “checked” in a note.

Investigate. These are teaching labels, not universal software categories. Preserve the reason and evidence for a decision.

Read / resources. Proofreading tutorials, page 78

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Connections and conclusions

PAGE 33 · Nearby branches

Explore. Cross two strings. Have they become one string?

Investigate. Spatial proximity alone does not establish a synapse.

Read / resources. [2]

PAGE 34 · Evidence of a synapse

Explore. Compare a labeled synapse diagram with a research image.

Investigate. Chemical synapses are identified using characteristic ultrastructure and annotation criteria. A colored dot is only a symbol.

Read / resources. [2], [5]

PAGE 35 · The pattern emerges

Explore. Trace both routes. Mark any part the pictures cannot settle.

Investigate. Checking one example establishes a local observation, not a general wiring rule. Keep damaged regions separate from supported connections.

Read / resources. [4], [5]

PAGE 36 · More than a pattern

Explore. Imagine the same pattern with a slow connection or a connection that quiets another cell. Could it behave differently?

Investigate. Cell identity, synaptic effects, timing, strength, and location affect function. A simple drawing leaves these properties out.

Read / resources. [13], [19], [20]

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Sharing and continuing

PAGE 37 · Variation and disease

Explore. List three reasons two maps might differ.

Investigate. Separate technical error and sampling from biological variation. Disease associations require appropriate comparison groups; causal claims need additional evidence.

Read / resources. [10], [11]

PAGE 38 · A claim others can check

Explore. Write an observation, a hypothesis, and a remaining question.

Investigate. Record image evidence, reconstruction version, and the limits of inference.

Read / resources. [7], [13]

PAGE 39 · Finishing an investigation

Explore. Name a question you answered and one that remains.

Investigate. An investigation can finish while the larger problem remains open. Resources may support questions their creators did not anticipate.

Read / resources. [5], [6]

PAGE 40 · Your next question

Explore. Choose a question small enough to investigate by observation.

Investigate. Name needed data, a possible source of error, and a result that would make you revise your explanation.

Read / resources. Training tracks and paper explorer, page 78

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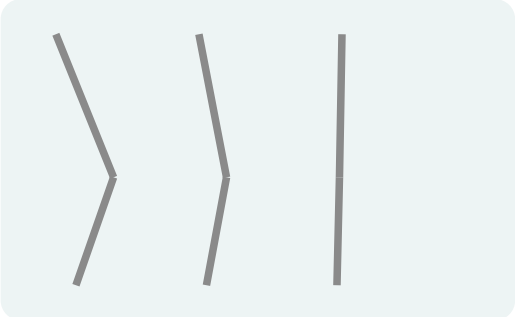
Try a mapping investigation

YOU NEED
Paper, colored pencils, tracing paper, and a partner.

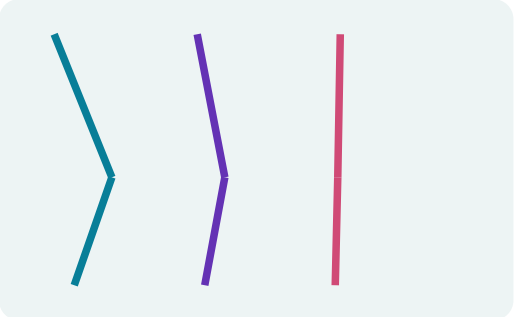
1. Draw winding shapes that pass close together.
2. Ask your partner to trace and color each shape.
3. Compare. Did a label join two shapes or split one?
4. Hide a small region. Can you know what is missing?
5. Report your checks, corrections, and uncertainties.

Switch roles. This is a learning model, not a test of readiness to proofread research data.

Original shapes



Labels to check



Compare each label with the original. Color is an annotation, not the cell's real color.

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A paired reading for a research group

STORY PAGES 29–32

Read the mystery twice

First read story pages 29–32 without technical discussion. Then revisit them as a reconstruction problem. Identify the observation, competing explanations, available evidence, and the difference between an edit and an unresolved case.

Make the decision independently

With an appropriately selected training example, ask each participant to propose an action and explain the evidence. Record uncertainty before discussion. Compare with an adjudicated reference that includes a rationale and acknowledges ambiguity. Do not use an unreviewed group majority as the answer key.

Test the downstream consequence

Specify one scientific analysis that could be affected by the decision. Compare its result under defensible alternatives. Discuss whether the uncertainty changes the conclusion or instead limits its scope.

Write the claim

End with three short statements: what the evidence supports, what it does not resolve, and what additional observation would help. This exercise illustrates a way to connect annotation practice with scientific reasoning; it is not a validated assessment of professional competence.

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Technical companion

APPENDIX A / SCIENTISTS AND ADULT READERS / STORY PAGES 13–40

A second reading of the adventure

The story asks how evidence becomes a scientific claim. This appendix follows the same decisions at a technical level: specimen preparation, image acquisition, registration, segmentation, synapse annotation, proofreading, and inference. Its workflow is illustrative, not a universal laboratory protocol.

Three objects to keep distinct

The specimen is biological material. The image volume is a measurement produced by preparation and acquisition. The reconstruction is an inferred representation derived from those images. A clean reconstruction can still reflect missing data, systematic preparation effects, or model error.

How to use the references

References [1]–[6] are on pages 66–68. Additional references [7]–[9] appear on page 69. Recommendations here are proposed research and training practices, not a validated educational intervention or a certification standard.

Navigation

Research checklist 56; scientific claims 57; proofreading 58; preparation 59; alignment 60; segmentation 61; synapses 62; patterns and variation 63; provenance 64; calibrating scientific expertise 65; references 66–76.

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A research review checklist

STORY PAGES 13–40

Question and data

What claim is being tested? What population and structures are observed? Which specimens, regions, and cases are excluded? Are the images sufficient for the decisions the analysis requires?

Reconstruction and validation

What counts as a split, merge, synapse, or graph edge? Who established the reference, and how were disagreements handled? What units, denominators, and sampling scheme underlie each reported metric? What remains unreviewed?

Human and automated decisions

Which decisions are automated, reviewed, or deferred? Is validation independent of training? Is performance measured on new cases? Are uncertainty and escalation treated explicitly rather than forced into a correct-or-incorrect label?

Inference and sharing

Does the conclusion survive plausible reconstruction or analysis changes? Are structure, function, and causality distinguished? Can another team identify the exact dataset state and reproduce the cohort and analysis? Can readers find the evidence behind the claim?

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From a map to a scientific claim

STORY PAGES 35–40 / REFERENCES [2], [5], [6]

Define what you want to estimate

Specify the population, region, cell types, and property being estimated. Separate a claim about observed reconstructed cells from a claim about an organism, species, or general wiring rule. Incomplete arbors and volume boundaries can affect which cells and connections enter the analysis.

Structure and function

A structural path supports a candidate route, not a measurement of signal flow under a particular condition. MICrONS [6] combines functional recordings with co-registered EM, enabling questions that neither modality alone resolves. Such integration still requires matching, selection, and measurement assumptions.

Sensitivity to reconstruction

As a proposed robustness check, repeat the relevant analysis across defensible alternative edits, exclusion rules, or annotation versions. Report whether the substantive conclusion changes. Propagate uncertainty where feasible and avoid treating all edges as equally certain when the evidence differs.

Scope and causality

Connectivity, activity correlations, and causal effects are distinct. Claims of causal influence require an appropriate experimental or inferential design beyond a static diagram. In the story, the crew supports a limited local claim and identifies the next measurement needed.

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Proofreading as an auditable decision

STORY PAGES 27–32 / REFERENCES [4], [5], [7], [8]

Before an edit

Identify the object, region, version, and scientific purpose. Inspect source images and relevant neighboring sections. Consider alternative explanations, including a volume edge, ambiguous continuity, registration problems, and segmentation error.

The decision record

Store the evidence inspected, the proposed correction, its rationale, the reviewer or process responsible, and any unresolved ambiguity. Distinguish an unchecked region from a checked but indeterminate one. The labels used in the story are teaching conventions, not standard fields in every platform.

Review effort

Risk-based triage can prioritize consequential or suspicious cases. It does not estimate residual error by itself. Audit an appropriate sample outside the flagged set and use independent adjudication for disputed or high-impact decisions. Agreement between reviewers is not proof of correctness.

A stopping rule

Choose a stopping rule appropriate to the intended analysis, available validation, and remaining uncertainty. State what was not exhaustively reviewed. Stable results under plausible annotation changes are useful evidence of robustness, but do not establish that every object is correct.

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The specimen and the measurement

STORY PAGES 17–22 / REFERENCES [1], [2], [6]

Preparation defines observability

Fixation, staining, embedding, and sectioning affect membrane continuity, contrast, and traceability. The relevant question is whether the resulting measurements preserve the structures needed for the intended analysis. Recording preparation conditions and local defects supports later interpretation.

Sampling and acquisition

Record voxel dimensions, section thickness where relevant, acquisition modality, image processing, and the spatial extent actually observed. Anisotropic sampling and missing sections can make some trajectories harder to follow. Nominal resolution alone does not establish that a particular boundary or synapse is identifiable.

Failure versus absence

A feature not detected in the image is not automatically absent from the specimen. Distinguish insufficient contrast, acquisition failure, preparation damage, volume boundaries, and a supported biological termination. Retained serial sections may permit reimaging; destructive acquisition and specimen loss impose different limits.

Review question

For the claim being made, which structures must be visible, and where does the dataset fail that requirement? Preserve defect locations so downstream analyses can exclude, stratify, or explicitly model affected observations.

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Registration and geometric fidelity

STORY PAGES 23–24

The inference

Registration estimates transformations that place images into a common coordinate system. Correspondences can be incomplete or wrong. Neighboring sections also differ because they sample distinct tissue planes, rather than repeated views of identical anatomy.

The tradeoff

Flexible transformations can improve local correspondence while introducing deformation. Saalfeld and colleagues describe elastic reconstruction with constraints on artificial deformation. Visual continuity should be evaluated alongside appropriate geometric and correspondence checks.

Suggested checks

Inspect difficult regions as well as easy landmarks. Track missing sections, folds, tears, and boundary effects. Examine whether a transformation creates implausible changes in local geometry. Keep transformation parameters and versions with the volume so coordinates and annotations remain interpretable.

Consequence for analysis

Geometric distortions may affect morphology, distances, and apparent continuity. The acceptable error depends on whether the analysis concerns topology, geometric measurements, or another endpoint. Do not replace an unresolved correspondence with a visually convenient trajectory.

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Segmentation and reconstruction error

STORY PAGES 25–26, 29–32 / REFERENCES [4], [8]

Representation

Segmentation labels image regions. Cell reconstruction may involve further grouping, tracing, and editing. A merge assigns material from distinct cells to one reconstructed object; a split divides one cell among multiple objects. Neither is equivalent to a biological fusion or physical break.

Evaluation units

Voxel overlap, object identity, traced path continuity, and graph connectivity are different evaluation targets. A metric dominated by large, easy regions can conceal a small error with substantial topological impact. Report what was counted and how reference decisions were obtained.

Dataset dependence

Flood-filling networks [4] demonstrate an approach to reconstruction, not a universal accuracy guarantee. NEURD [8] provides automated proofreading and feature extraction with evaluated correction performance. Its results also illustrate why performance and downstream findings should be interpreted within the dataset and proofreading state used.

Suggested validation design

Separate development and evaluation cases. Sample challenging and representative regions, document inclusion rules, and audit both flagged and unflagged cases. Prefer held-out specimens or regions when shared morphology or imaging conditions would otherwise inflate apparent generalization.

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Synapses and graph construction

STORY PAGES 15–16, 33–36 / REFERENCES [2], [5], [9]

Proximity is insufficient

Branches passing near one another do not establish synaptic connectivity. In EM-based work, synapse calls depend on recognizable ultrastructure and defined annotation criteria. Kasthuri and colleagues [2] explicitly examine the limits of proximity as a predictor.

Several decisions, several errors

Detection, partner assignment, and direction assignment are distinct tasks. A correctly detected site with the wrong partner still yields an incorrect graph edge. The graph definition must also specify whether edges are binary, weighted by contact counts, or constructed using another rule.

Report precision and recall

TP means true positives (correct detections), FP false positives (incorrect detections), and FN false negatives (missed detections). For a defined event and reviewed reference: precision = TP / (TP + FP); recall = TP / (TP + FN). Report counts, matching tolerances, sampling, and uncertainty. Do not transfer a score between site-level and connection-level evaluations without justification. SynEM [9] evaluates automated detection and partner assignment.

Interpretation

A synapse count is a structural quantity. It is not automatically a measured physiological weight or effective influence. State which synapse types and structures are covered and what remains outside the annotation procedure.

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Patterns, variation, and impact

Circuit motif and our setting

Our fictional expedition is in mouse hippocampus. A, B, and C are teaching symbols, not identified cell types or a published discovery. Real circuit descriptions include cell identity, synaptic sign and strength, dendritic location, and dynamics. Repetition alone does not establish statistical enrichment; define sampling, edges, and a null model. Compare prior hippocampal experiments [19], [20].

Computing primitive

A proposed basic operation, such as combining inputs or detecting a change. Topology alone does not identify the operation. Synaptic sign, strength, timing, cell properties, and state matter. Use measurements and experiments to distinguish mechanisms [13].

Connectopathy and normal variation

Connectopathy is a framing for disorders involving disrupted connectivity, not a diagnosis inferred from an unusual motif. A reference dataset is not a universal healthy template. Compare appropriately matched individuals and account for technical uncertainty [10], [11].

Efficiency, robustness, and translation

Efficiency means achieving a defined outcome with fewer resources. Robustness means maintaining performance under specified disturbances. Both need task-specific tests. Health translation connects research findings to evaluated clinical benefit; the story demonstrates no treatment or diagnostic test.

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Provenance and reproducible analysis

STORY PAGES 38–40 / REFERENCES [5]–[7]

A changing dataset

Proofreading changes reconstructed objects and may change the assignment of annotations to cells. A dataset name or a current object identifier is therefore insufficient to specify the exact state used in an analysis.

Version-aware infrastructure

CAVE [7] supports proofreading, annotation, and analysis at specified time points. Its purpose is especially relevant when distributed collaborators continue editing while analyses are underway. The analysis must still record the release or materialization state and the queries actually used.

Suggested minimum record

Record dataset and release, timestamp or materialization version, relevant identifiers, coordinate conventions, query and filtering code, annotation definitions, and edit or exclusion policies. Archive enough information to reconstruct cohort selection as well as final summary figures.

Communicating an update

When a result changes, identify whether the cause was revised biological interpretation, corrected reconstruction, a different cohort, or an analysis change. Preserve the earlier result and its provenance rather than silently substituting a newer map.

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Calibrating scientists

SCIENTIFIC EXPERTISE ACROSS THE RESEARCH PROCESS / PROOFREADING AS ONE EXAMPLE

Calibrating scientific expertise

Calibrating scientific expertise aims to catalyze the transformation of raw talent into scientific capability. Through practice, feedback, and mentoring, learners align judgment with evidence: asking useful questions, choosing methods, testing explanations, interpreting results, and communicating defensible claims. Proofreading is one special case. The broader goal is independent scientists who know how to investigate—and when their evidence is not enough.

Different abilities need different checks

Confidence calibration compares stated confidence with observed correctness. Agreement measures consistency between people. Scientific expertise also requires sound study design, inference, and explanation. No single score establishes expertise; reference judgments can themselves be uncertain.

Practice across the research process

Use cases that require learners to frame a question, select a measurement, identify a flaw, interpret an unexpected result, or explain a limitation. Obtain independent reasoning, give specific feedback, and assess new cases. In proofreading, this can mean checking a boundary; in analysis, it can mean choosing a fair comparison or recognizing an unsupported causal claim.

Learning engineering and transfer

Learners improve their reasoning through feedback. Educators use evidence about learning to improve instruction and assessment: this is learning engineering. Test whether skills transfer to unfamiliar data, questions, and settings. These are proposed educational practices, not a claim that this book has demonstrated their effectiveness.

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Research behind the journey

Preparation and reconstruction

[1] Hua, Y., Laserstein, P., and Helmstaedter, M. (2015).

Large-volume en-bloc staining for electron microscopy-based connectomics. Nature Communications, 6, 7923.

A methods paper about staining suitable for tracing larger tissue volumes. Start with figures comparing preparation quality.

https://doi.org/10.1038/ncomms8923

[2] Kasthuri, N., and colleagues (2015).

Saturated Reconstruction of a Volume of Neocortex. Cell, 162(3), 648–661.

Kasthuri, Lichtman, and collaborators reconstructed a small mouse-cortex volume. Relevant to distinguishing nearby branches from actual synaptic connections.

https://doi.org/10.1016/j.cell.2015.06.054

These papers support the scientific methods and examples in the guide. They do not report the fictional adventure or establish this book's educational effectiveness.

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Research behind the journey

Alignment and automation

[3] Saalfeld, S., Fetter, R., Cardona, A., and Tomancak, P. (2012).

Elastic volume reconstruction from series of ultra-thin microscopy sections. Nature Methods, 9, 717–720.

A method for reconstructing volumes from serial sections while controlling artificial deformation.

https://doi.org/10.1038/nmeth.2072

[4] Januszewski, M., and colleagues (2018).

High-precision automated reconstruction of neurons with flood-filling networks. Nature Methods, 15, 605–610.

Research on automated segmentation and evaluation. Examine how the authors define and measure errors.

https://doi.org/10.1038/s41592-018-0049-4

These papers support the scientific methods and examples in the guide. They do not report the fictional adventure or establish this book's educational effectiveness.

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Research behind the journey

Maps and discovery

[5] Dorkenwald, S., and colleagues (2024).

Neuronal wiring diagram of an adult brain. Nature, 634, 124–138.

Dorkenwald, Seung, Jefferis, and the FlyWire collaboration: an adult female fruit-fly brain wiring diagram. Explore reconstruction, proofreading, annotation, and shared tools.

https://doi.org/10.1038/s41586-024-07558-y

[6] The MICrONS Consortium (2025).

Functional connectomics spanning multiple areas of mouse visual cortex. Nature, 640, 435–447.

Combines functional measurements with electron-microscopy reconstruction. A starting point for asking how wiring relates to activity.

https://doi.org/10.1038/s41586-025-08790-w

These papers support the scientific methods and examples in the guide. They do not report the fictional adventure or establish this book's educational effectiveness.

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Additional technical literature

REFERENCES [7]–[9] / COMPLEMENTS PAGES 54–56

[7] CAVE: Connectome Annotation Versioning Engine

Nature Methods 22, 1112–1120 (2025). Infrastructure for version-aware proofreading, annotation, and analysis. DOI: 10.1038/s41592-024-02426-z

[8] NEURD offers automated proofreading and feature extraction for connectomics

Nature 640, 487–496 (2025). Automated reconstruction correction and feature extraction with validation and dataset-specific limitations. DOI: 10.1038/s41586-025-08660-5

[9] SynEM, automated synapse detection for connectomics

eLife 6, e26414 (2017). Automated synapse detection and partner assignment, with evaluation at explicitly defined units. DOI: 10.7554/eLife.26414

Reading strategy

For each paper, identify the data, decision being evaluated, reference construction, metric denominator, and scope of the conclusion. Compare these before comparing headline performance numbers.

https://doi.org/10.1038/s41592-024-02426-z

https://doi.org/10.1038/s41586-025-08660-5

https://doi.org/10.7554/eLife.26414

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Research behind the journey

Health and biological variation

[10] NIH BRAIN Initiative

Program overview and research purpose.

The motivation for understanding circuits and developing tools that can support progress on brain disorders; not evidence that this fictional motif diagnoses disease.

https://www.nih.gov/brain

[11] Witvliet, D., and colleagues (2021).

Connectomes across development reveal principles of brain maturation. Nature, 596, 257–261.

Primary research comparing eight genetically matched C. elegans individuals across development. Supports attention to developmental and individual variation.

https://doi.org/10.1038/s41586-021-03778-8

The story's motif and its discovery are fictional. These resources support the questions and methods, not a reported clinical finding.

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Research behind the journey

Energy and computational predictions

[12] Levy, W. B., and Calvert, V. G. (2021).

Communication consumes 35 times more energy than computation in the human cortex, but both costs are needed to predict synapse number. PNAS, 118, e2008173118.

An energy-budget analysis. The approximate 20 W brain budget includes more than computation and is not a data-center benchmark.

https://doi.org/10.1073/pnas.2008173118

[13] Lappalainen, J. K., and colleagues (2024).

Connectome-constrained networks predict neural activity across the fly visual system. Nature, 634, 1132–1140.

Primary modeling research combining connectivity and task constraints to predict activity; it does not show that wiring alone determines function.

https://doi.org/10.1038/s41586-024-07939-3

[25] Herculano-Houzel, S. (2011).

Scaling of brain metabolism with a fixed energy budget per neuron. PLOS ONE, 6, e17514.

Comparative analysis providing context for the adult human brain’s share of body mass and resting energy use.

https://doi.org/10.1371/journal.pone.0017514

The story’s motif and its discovery are fictional. These resources support the questions and methods, not a reported clinical finding.

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Research behind the journey

An agenda and a way to learn

[14] Zador, A., and colleagues (2023).

Catalyzing next-generation Artificial Intelligence through NeuroAI. Nature Communications, 14, 1597.

A research agenda motivating biological inspiration for AI. Treat proposed future benefits as goals, not established outcomes.

https://doi.org/10.1038/s41467-023-37180-x

[15] NeuroTrailblazers / Nanoscale.

Why Map the Brain and The Neuronauts Expedition.

The site provides the educational framing and resource pathway. Consult primary papers for individual scientific claims. Accessed 3 November 2026.

https://www.neurotrailblazers.org/technical-training/01-why-map-the-brain/

The story's motif and its discovery are fictional. These resources support the questions and methods, not a reported clinical finding.

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Research behind the journey

Earlier maps and complementary tools

[16] White, J. G., Southgate, E., Thomson, J. N., and Brenner, S. (1986).

The structure of the nervous system of the nematode Caenorhabditis elegans.

Foundational reconstruction of the worm nervous system. Philosophical Transactions B, 314, 1–340.

https://doi.org/10.1098/rstb.1986.0056

[17] Winding, M., and colleagues (2023).

The connectome of an insect brain. Science, 379, eadd9330.

Whole larval Drosophila brain reconstruction, from the Zlatic and Cardona teams and collaborators.

https://doi.org/10.1126/science.add9330

[18] Van Essen, D. C., and colleagues (2013).

The WU-Minn Human Connectome Project: An overview.

Human imaging complements synapse-scale reconstruction. NeuroImage, 80, 62–79.

https://doi.org/10.1016/j.neuroimage.2013.05.041

Selected primary sources behind the timeline and the choice of methods.

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Research behind the journey

Hippocampal clues and open questions

[19] Bloss, E. B., and colleagues (2018).

Single excitatory axons form clustered synapses onto CA1 pyramidal cell dendrites. Nature Neuroscience, 21, 353–363.

The Spruston team and collaborators connect fine anatomical patterns with questions about dendritic integration. Location and multiple synapses matter beyond a simple edge.

https://doi.org/10.1038/s41593-018-0084-6

[20] Hippocampal feedforward inhibition (2013).

Hippocampal feedforward inhibition focuses excitatory synaptic signals into distinct dendritic compartments. PLOS ONE, 8, e80984.

Experiments in mouse slices illustrate why recordings and cell properties are needed to interpret a wiring pattern.

https://doi.org/10.1371/journal.pone.0080984

The fictional A–B–C example is not a reconstruction of these experiments. Prior findings suggest hypotheses to compare, not an answer to copy.

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Research behind the journey

Tools that opened new windows

[21] Denk, W., and Horstmann, H. (2004).

Serial block-face scanning electron microscopy to reconstruct three-dimensional tissue nanostructure. PLOS Biology, 2, e329.

An important volume-imaging method; an alternative to collecting serial sections.

https://doi.org/10.1371/journal.pbio.0020329

[22] Briggman, K. L., Helmstaedter, M., and Denk, W. (2011).

Wiring specificity in the direction-selectivity circuit of the retina. Nature, 471, 183–188.

An example of relating synaptic wiring to a circuit computation.

https://doi.org/10.1038/nature09818

A selected reading path, not an exhaustive history. Advances depend on experimentalists, engineers, annotators, and shared infrastructure.

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Research behind the journey

Building shared maps at scale

[23] Zheng, Z., and colleagues (2018).

A Complete Electron Microscopy Volume of the Brain of Adult Drosophila melanogaster. Cell, 174, 730–743.e22.

Bock and collaborators created the whole-brain image resource underlying subsequent reconstruction work.

https://doi.org/10.1016/j.cell.2018.06.019

[24] Scheffer, L. K., and colleagues (2020).

A connectome and analysis of the adult Drosophila central brain. eLife, 9, e57443.

The hemibrain collaboration brought together Janelia and Google contributions to imaging, reconstruction, and analysis.

https://doi.org/10.7554/eLife.57443

Read these alongside FlyWire [5] and MICrONS [6]. These are complementary achievements across species, scales, and methods.

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My next question

What do I want to find out?

What could I observe?

What might fool me?

What would make me change my mind?



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Continue your expedition

Read and explore with the crew

neurotrailblazers.org/neuronauts/

Neuronauts Junior Lab

neurotrailblazers.org/neuronauts/kids/



neurotrailblazers.org

EM Proofreading Tutorials

neurotrailblazers.org/technical-training/proofreading-tutorials/

Training Tracks

neurotrailblazers.org/tracks/

Research Papers

neurotrailblazers.org/technical-training/journal-club/

Paper Explorer

neurotrailblazers.org/technical-training/journal-club/graph/

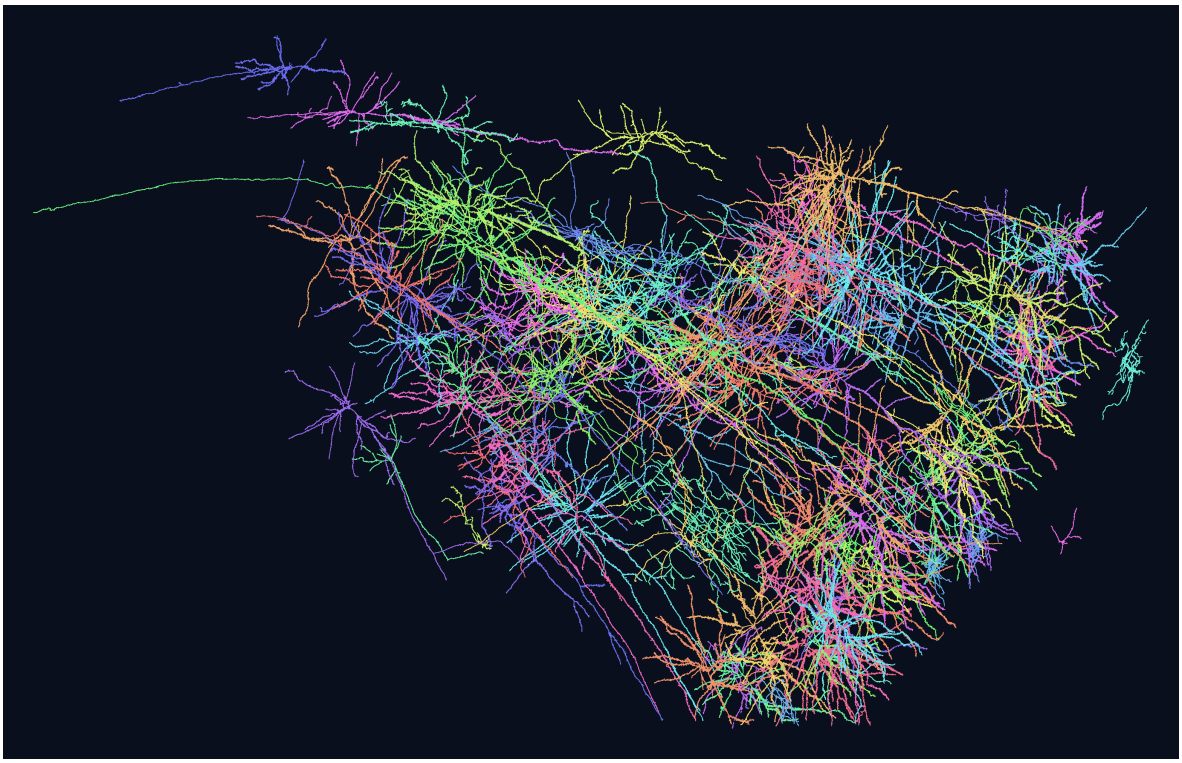
Online resources can change. Use the paper titles and persistent DOI links in the bibliography to locate research independently.

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A real frontier inside us



These traces come from H01's released set of 104 proofread neurons in a small sample of human cortex. Each color identifies a different neuron—not a cell type.

This flat view preserves the positions of their traced branches while looking along one axis. Crossing lines do not establish a synapse. This is human cortex, distinct from our story's mouse hippocampus.

Data: H01, Lichtman Lab (Harvard University) & Google Research. Original renderings for Gray-Roncal & Floryanzia (2026). Data and renderings: CC BY 4.0.

H01: Shapson-Coe et al., Science (2024), doi:10.1126/science.adk4858

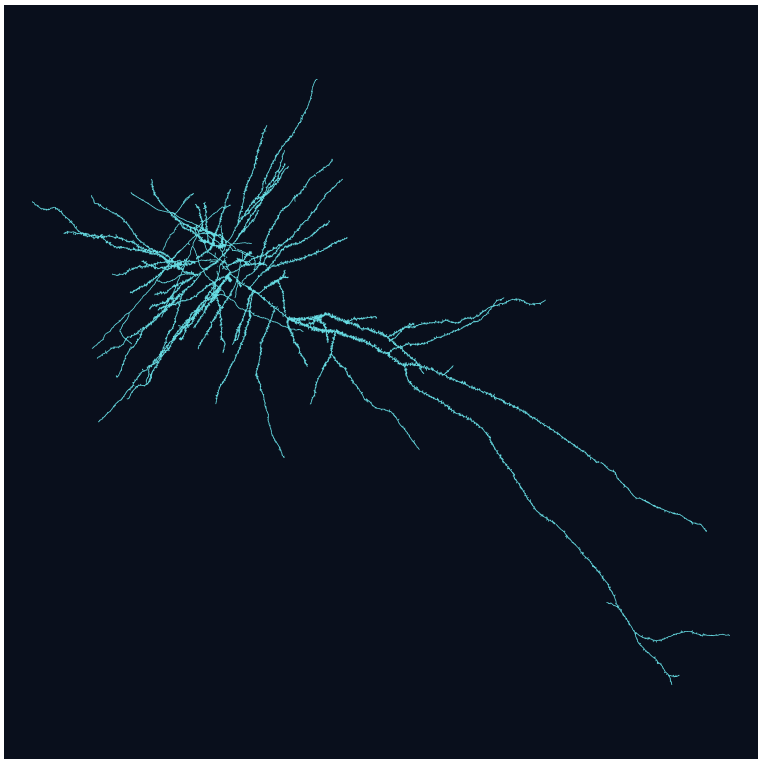
Data and these renderings: CC BY 4.0 · creativecommons.org/licenses/by/4.0/

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One neuron, many branching paths



Follow the branches of one reconstructed H01 neuron: cell 3955003482. These lines trace its shape; they do not show the thickness of its branches or identify its synapses.

We created this flat view from the released reconstruction. Colors and line widths are display choices. Even one cell has far more structure than the circles in our teaching diagram.

Data: H01, Lichtman Lab (Harvard University) & Google Research. Original renderings for Gray-Roncal & Floryanzia (2026). Data and renderings: CC BY 4.0.

H01: Shapson-Coe et al., Science (2024), doi:10.1126/science.adk4858

Data and these renderings: CC BY 4.0 · creativecommons.org/licenses/by/4.0/

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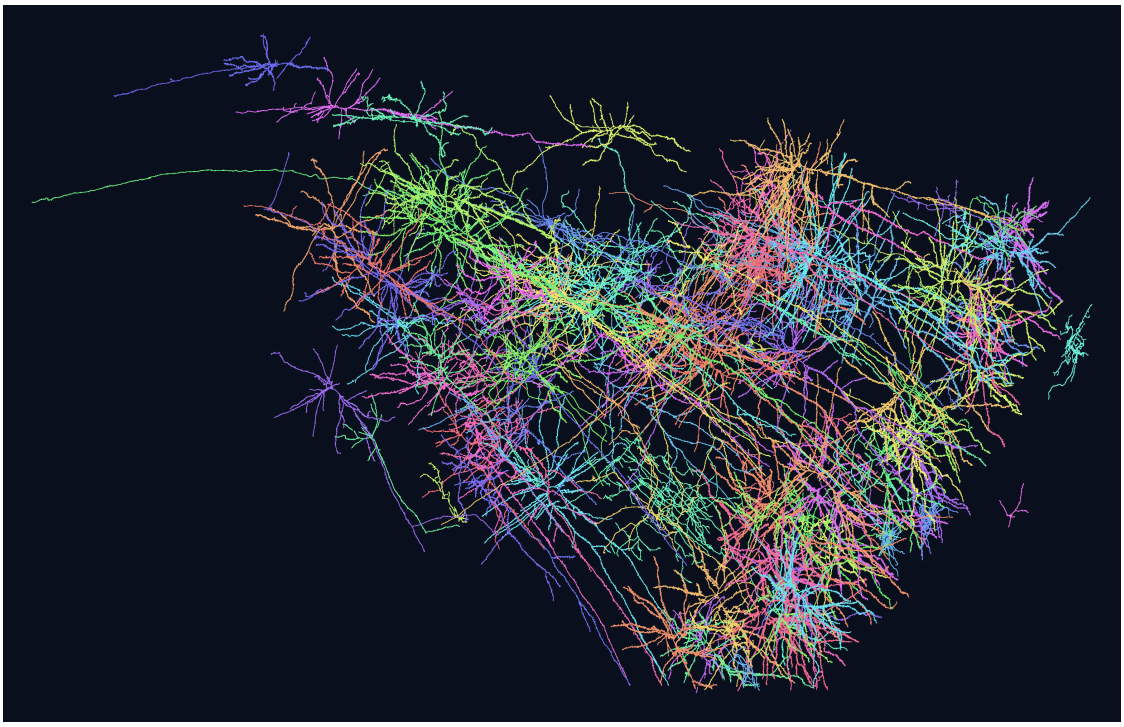
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A tiny connection.
A question that matters.



How do we map a brain—and know our map is right?

Join the Neuronauts as they follow a puzzling wiring pattern, check their tools, and learn how trustworthy discoveries begin. A story for curious readers, with deeper science for those ready to explore further.

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H01 data: Lichtman Lab (Harvard University) & Google Research. Shapson-Coe et al. (2024).
Original rendering for Gray-Roncal & Floryanzia (2026). Data and rendering: CC BY 4.0.

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