The State of Brain Emulation Report 2025

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Funded by Fieldcrest Foundation and Foresight Institute

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Preface

This report started as a long list of notes. The authors were working on a blog post series on brain emulation and a review paper on scaling current data acquisition efforts up to human levels. Over time the notes evolved into more and more of a textbook character until we eventually decided to transition them into a proper report.

Building on the theoretical framework established by Bostrom et al. (2008), this report marks a transition from speculative brain emulation roadmaps to empirical reality in the field neuroscience. Brain emulation—the creation of digital copies of biological brains—has

emerged as one of the most ambitious scientific endeavors of our time and subfields delivered many concrete achievements in the past decades.

This inaugural “State of” report provides a comprehensive overview of the field's current state, recent advances, and future challenges, intended for researchers, funders, and technical professionals seeking deep understanding of the field. It aims to provide clarity on capabilities, trends and results across a field that is extremely interdisciplinary. Hopefully future versions can update the respective sections according to scientific progress and provide interested audiences with a continuously updated overview.

We encourage readers to engage with the - likely more accessible - supplementary material that comes with this report:

- The initially planned blog series, published in collaboration with Asimov press [WIP]
- The highly visual, plain language summary poster, that synthesizes the main findings accessible for laymen audiences
- Data repositories that include quality controlled data sets used to generate figures for this report
- A calculator for the costs and timelines of brain emulation initiatives we build on top of the data [WIP]

The report does not claim completeness. For instance, our research provides only a glimpse into neuromodulators or neuroplasticity, and completely omits a discussion of transcriptomics, or proteomics. As a primarily technical document, we also did not examine ethical and social considerations. We took the liberty to discuss these speculative ethical and social issues in a set of narrative vignettes in the blog post series, for those who are interested.

We hope the report will help to have constructive conversations between the various communities, from neuroscientists to investors.

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Executive Summary

To be written - but check-out the poster!

Link to comment:

https://acrobat.adobe.com/id/urn:aaid:sc:AP:301448ee-f236-40c8-9a51-959dcbc53827

Quick Video: https://www.loom.com/share/d3e54e1fcc3f47a59852ad0e5ebe4c58

Status of Brain Emulation Report 2025 - Summary

Full report at https://[TBD].org | Supported by Fieldcrest Foundation & Foresight Institute

KEY TAKEAWAYS

1 The Field
The brain emulation adjacent field likely consists of less than 5,000 people globally who operationalize approx. $1B/year Resources are highly dependent on a few major funders across government (60%), industry (20%) and philanthropy (20%). Given the field's size, individual contributors joining or leaving matters.

2 Collaboration
Substantial efforts and consortia like FlyWire have connected members of different fields. Still, these efforts are decentralized across institutions. Further integration of interdisciplinary efforts promise faster feedback loops and eventually better outcomes

3 Data
Peta-scale datasets exist, increasingly standardized. Individual methods fail to capture all dimensions of an organisms' neurobiology (e.g. high spatial and temporal resolution). No whole brain recordings exist (>90% of neurons) in any organism. Freely moving behavior is rare. Connectomes currently lack molecular annotation.

4 Progress
Since the 1980s, both neuralactivity recording and synaptic resolution brain mapping capabilities increased exponentially by three to five orders of magnitude. Computational requirements are increasingly met by single devices rather than data centers. Structure to function extrapolation has not yet been demonstrated.

5 Unknowns
The significance of various factors such as neuromodulation (hormones, glia cells, etc), neuroplasticity, gap-junctions, or molecular annotation of various proteins remain unknown. For most of these, no major initiatives try to elucidate their relevance systematically.

6 Opportunities
Many tractable opportunities could advance the field meaningfully and need both funding and talent. Attracting large-scale investment mandates overcoming the pre-paradigmatic state and demonstrating fraction meaningful traction towards substantial economic benefits on a societal and venture capital level.

THIS IS A DRAFT DO NOT SHARE WITHOUT PERMISSION!

Milestones & Funders

Model Organisms for Brain Emulation

C. elegans
Anatomy 1mm worm, 302 neurons (half "brain", half ganglia). Neurons communicate with spikes and graded signals. Lifespan: 15 days

Behavior crawling, reversals, following along chemical gradients Association of cues to food or harm

Larval Zebrafish
Anatomy ~5mm long, 0.1 mm3 brain volume with ~ 100,000 neurons, X neurons in the spine, action potentials up to 80Hz, transparent up to 20d

Behavior stereotyped swimming movements, reflex response to visual, acoustic / vibrational, chemical and tactile stimuli, aggregation with other larvae with preferences for partners, circadian rhythms, Habituation to repeated stimuli

Fruitfly
Anatomy 3mm long, 0.08 mm³ brain volume 140k, ventral chord 15k neurons, neural structures enclosed by partially transparent exoskeleton, neural tissue has air-filled tubes avg. age 50d

Behavior Complex movements, incl. flight, precise grooming, fight and defense (boxing and fencing); elaborate courtship rituals; non-associative learning, aversive courtship memories after rejection, recall outcomes of past aggressive encounters, behavior changes based on social context

Mouse
Anatomy ~25-35g Lifespan: 2–3 years. Brain volume: ~450mm³ (~63–70 million neurons) Spinal cord: ~8 million neurons. Neuronal activity: median firing rate ~25 Hz; fast-spiking interneurons can exceed 200 Hz

Behavior Extensive cognitive abilities, stable individual differences (e.g., in anxiety and sociability). Complex social organization with hierarchies and communication via scent marking / vocalizations. Elaborate courtship and maternal behaviors.

Human
Anatomy 15cm long, 1.5L of brain, ~86B neurons (80% in Cerebellum), XB neurons in the spine.

A variety of other model organism exist: Danionella, bees, cockroaches, rats, non-human primates. The report's scope was limited to the model organisms mentioned here.

Multimillion Dollar neuroscience initiatives in government, industry and philanthropy plotted against other megaprojects. Nearly all neuroscience initiatives began in the past ~10y. This points to the recent advances that enabled large-scale brain mapping, such as imaging, sequencing, and computing, but also to the pre-paradigmatic state.

Neurodynamics

Methods and trajectories recording neuronal activity

Neurodynamics focuses on capturing neural activity as it unfolds in real time and how it generates behavior. **(Left)** Neural activity can be captured with non-invasive and invasive methods. Non-invasive methods (e.g., fMRI, functional ultrasound) excel at recording from deeper brain regions over longer periods but sacrifice spatial resolution and only capture aggregate neural activity. In contrast, invasive methods (e.g., multi-electrode arrays, calcium or voltage imaging) offer single-neuron spatial precision and rapid sampling rates at the millisecond scale. They are currently not able to scale beyond ~1mm³ and require surgical intervention and even genetic modification. The field has seen remarkable progress, with the number of simultaneously recorded neurons doubling approximately every 5.2 years, reminiscent of Moore's law. **(Right)** Illustration of the tradeoffs across modalities and the relative information theoretic value / cost per Terabyte of data.

Existing neural recordings in fixated organisms

Radar plots based on the aggregation of the most advanced recordings in the past decade. The outer ring is normalized to the maximum known values in the respective organism (blue label). Each ring represents one order of magnitude distance from the maximum. Dimensions include: spatial resolution, brain volume, temporal resolution, and individual / cumulative recording duration. The only datasets in fixated organisms shown (data for freely moving organisms only available for C.elegans and mice).

No recordings for any neuromodulators are plotted. [current plots are not optimized for this poster yet]

Heatmap plot of significant brain recording publications across different organisms. The data is the same as in the radar chart, but broken down by author.

Note that often data was lacking and a best-guess approach was followed as there wasn't yet the time to go through all the publications in detail.

Connectomics

Methods and trajectories recording neuronal activity

Connectomics aims to reconstruct all neuronal connections—collectively called the connectome—at ultrasmall scales of around 10–20 nm resolution. **(Left)** The workflow begins with sample preparation, which can involve staining (e.g., osmium, ariltbody labeling), tissue expansion, or genetic engineering. Brain tissue is then cut into pieces typically smaller than 1 mm³ for scanning on electron microscopes (costing $1–4M) or lightsheet microscopes ($0.1–0.5M). To date, all large-scale connectome projects have relied on electron microscopy with osmium staining, though newer methods like expansion microscopy and genetic barcoding are now demonstrating feasibility. Scanning at 10–20 nm resolution produces petabytes of image data per cubic millimeter of tissue. Once collected, these data must be aligned, traced, and proofread—steps whose cumulative costs often rival or exceed the initial scanning. Fortunately, algorithmic advances are driving down these post-processing expenses. **(Right)** Exponential improvements over time in the number of reconstructed neurons per $1M, as achieved by four major connectomics projects.

Existing neuron reconstructions at synaptic resolution

10 complete connectomes, one composite, including both sexes and five different developmental stages (EM). Synaptic resolution. Ongoing efforts with expansion microscopy.

One full brain and 10% of spinal chord in individuals prior sex differentiation. Synaptic resolution via EM. Expansion efforts ongoing.

One full brain, one half brain, one ventral nerve cord connectome, all in different female individuals. Synaptic resolution via EM. Expansion and X-ray efforts ongoing.

1mm3 male mouse brain cortex (0.2% total brain volume). Synaptic resolution. Expansion, barcoding and X-ray efforts ongoing.

1mm3 female human brain cortex (0.0001% total brain volume). Synaptic resolution via EM.

Time series structural data on neuronal plasticity on larger volumes is not existant

Figure to illustrate the sizes of neurons, synapses and Gap junctions, Iascone, 2020, mouse primary somatosensory cortex

(A) Sparsely labeled layer 2/3 pyramidal neuron (PN). Scale bar: 500 µm. Inset, higher magnification of the neuron shown. (E) Neuron trace of (A) Scale bar: 50 µm. (F) volume from inset in (E) with identified inhibitory synapses labeled in white, corresponding trace, overlaid spine annotations, and inhibitory synaptic annotations associated with nodes within the trace. Scale bar: 2 µm.

Computational Neuroscience

Methods and trajectories recording neuronal activity

Computational Neuroscience uses neuroscientific datasets to model brain activity (simulation), and embody those simulations in a virtual or physical world (emulation). **(Left)** Simulation approaches differ in the number of parameters, data fitting methodology and the connectivity assumptions. In its simplest form, each neuron is represented by a few parameter which is set based on prior knowledge (e.g. Leak-integrated and Fire) randomly connected to others. More detailed approaches use hundreds of parameters per neuron and often subdivide them in compartments (e.g. Hodgkin Huxley) and leverage connectomes to determine the right connectivity. Data fitting can go up to large-data machine learning approaches. These simulations then control bodies and receive inputs (as cochlear implants encode external sound into neural signals) to verify organism behavior repertoires. **(Right)** Brain simulations computational demands (operations, fast storage and interconnect speed) depend primarily on the number of neurons and the above mentioned parameter count. Only recently is simulation of small scale brains possible on enduser devices. Mammalian models often still require data center access and even then face simulation penalties due to lack of sufficient shared fast storage or interconnect.

Existing brain emulations across 10 dimensions

Heatmap plot of significant brain simulations. The data and rating is the same as in the radar chart, but broken down by author.

Frontiers & Recommendations

	Fieldbuilding	Data	Technology	Infrastructure	Finance
$100k	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
$1M	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
$10M	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
$100M	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
$1B	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
$10B	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]

Sketch of a dedicated brain emulation facility that combines animal facilities, sample processing, structural and functional imaging with data centers and administrative areas. The scale of such a facility surpasses facilities like the newly built TSMC Arizona fab, but stays below Teslas Nevada Gigafactory.

Data Availability: xyz; Other additional notes: ...

Introduction

For this report we selected three major fields of neuroscience that guide the discussion around emulating brains: **neurodynamics**, **connectomics and neuroplasticity**, and **computational neuroscience**. Implicitly, we will also dive in many facets of Data Science, given the scale of data acquisition.

Neurodynamics explores neural activity patterns over time using both non-invasive and invasive methods. The former being fMRI and EEG, to collect large amounts of brain data *while organisms are alive.* The latter being calcium imaging, two-photon imaging or voltage methods, involving surgery for access to brain areas, genetic engineering and other preparation steps to then record from different brain areas electric and chemical inputs and outputs over time.

Connectomics charts the physical links among neurons, trying to reconstruct brain structures after an organism is deceased. Scientists begin by perfusing, extracting, and slicing the brain into small pieces, which can then be stained and scanned using microscopes. Every time a slice of the brain is imaged, the data must be checked, compressed, and stored. AI tools can be used to stitch together individual images to reconstruct neurons and their connections to each other.

Computational neuroscience takes all the collected data and derives models and simulations. They use the connectome graph, functional recordings, and neuronal plasticity data to "run" the brains on devices up to peta- to exascale supercomputers. Embodied simulations (which we will call emulations for the rest of the report) map brain-area activity onto digital or physical bodies and route sensory input back into the simulated brain. After fine-tuning of parameters through feedback loops from embodiment, whole-brain data recordings, and other mechanisms, verification tests the emulation against cognitive, behavioral and other benchmarks.

The following figure illustrates these steps

Figure Overview Using the full toolkit of neurodynamics to measure variables influencing brain activity data while the organism is still alive, then destructive reconstruction by connectomic methods to digitally represent the brain's structure, and finally computational neuroscience approaches to use all of the data to create faithful emulation that hopefully match success criteria.

1 Neurodynamics
Non-invasive methods for brain functional recordings
Invasive methods for brain functional & chemical recordings
Behavior & external stimuli / environment
2 Connectomics
Sample preparation & slicing
Scanning
Neuron & connectome reconstruction
3 Computational Neuroscience
Simulation
Embodiment
Behavior: rest / move / learn,...
Verification
Analysis & modelling

No consensus exists on what exactly constitutes a faithful brain emulation, or why it should be built in the first place. Some imagine it as a tool to deepen our understanding of the brain, or to develop new therapies, or simply to create a form of intelligence that more closely aligns with our own nature.

Even when reasonable alignment on the outcome exist, experts hold wide-ranging opinions on what *level* of biological detail we'll need to eventually successfully emulate a brain. Will we need to understand every neuron, or go all the way down to individual molecules? Will we need to emulate the whole body, the entire nervous system, the whole brain, or merely key parts of the brain — such as the cortex or cerebellum? Many factors could play a role in determining neuronal processing external to neurons (Glial cells, neuromodulatory peptides and hormones), about neurons (connections, cell types, synaptic activity, gap junctions, membrane molecules) down to the molecular level (ion concentrations, pH). We do not know what matters how much. And this report does not claim to cover every conceivably important datapoint.

While we won't cover every facet of biology, neuroscientists have priors. The decision to focus on neural activity data in conjunction with behavior and the structural connections between neurons in addition to some molecular annotation seem like a reasonable starting point. Eventually, what data is necessary is going to be a strictly empirical question that must be resolved via interplay between simulations and experiments in a virtuous self-correcting loop. All the aspects that are covered in the report are outlined in the following overview figure.

Overview of all the components discussed in the report. Brain emulation is modelling electrical neuronal activity constrained by its structure (connections between neurons, other cell types) and potentially substructure (individual transmitters and proteins, though other levels of detail are possible). During embodiment, brain activity then needs to get decoded in behavior such as language or movement, and encode information from the environment such as light and sound, but also local or body wide signal molecules like hormones or neuropeptides. This process needs to translate close to flawlessly between digital neuronal spikes and the physical world, very similar to what modern brain computer interfaces or cochlea implants are doing. And this interplay needs to capture accurately the changes in brain structure and function over time, i.e. brain plasticity.

Eventually, a faithful brain emulation represents a digital system that over extended periods of time; at least a few days, but ideally weeks or months behaves just like the biological organism (across most dimensions, what some authors call the "embodied" turing test) and deriving its behavior from the same underlying neuro-activity processes. In other words, we should not be able to distinguish a digital reconstruction from the monitored brain activity of a biological population.

Next to humans, the State of Brain Emulation Report 2025 currently orients around four model organisms: C.elegans, Fruitfly, Zebrafish Larvae, and Mouse. Many other model organisms exist, though these present the most prominent examples. For each, the report provides a detailed analysis of the current state of research, covering Neurodynamics (How we capture brain activity and function), Connectomics (How we map brain structure and connections), and Computational Neuroscience (How we simulate brain behavior). These organism chapters are complemented by general discussions of neuroscience methods and data science challenges.

In a last step, the report tries to offer a synthesis of eminent gaps and exemplary projects on the paths to more successful brain emulations. Here we also describe the motivations and past contributions of the field's three main funders (Government, Industry and Philanthropy).

We will start by introducing the organisms, their anatomy and behavior, but also what sets them apart as a promising model for eventual human scale brain emulation.

Definition of some terms

Simulation vs Emulation: The terms are ill defined and often used interchangeably. In the traditional sense the goal of simulation is to analyze, predict, or understand how a system works, whereas emulation allows software or hardware designed for one system to run on another system as if it were native. For this report we define computational models of the brain that do not have embodiment features (input, output, physical or virtual bodies) as simulation, those that have those features as emulation.

"Whole Brain": In the literature many things get called whole brain - from neuronal recording of a small percentage of neurons across the big parts of the brain volume, to actual single cell activity recording across all neurons of the organism. For this report we will define - somewhat arbitrary - as as capturing at least 90% of neurons of 90% of the brain volume.

...

The organisms

In this chapter we outline the key anatomical and behavioural features of the four model organisms we will revisit throughout the report: C. elegans, Larval Zebrafish, Drosophila, Mouse. Those constitute the four commonly used animal models that have been used in neuroscience. We aggregate interesting findings from other major model organisms in a "Miscellaneous Organisms" chapter and close with descriptions for humans.

Checklist for each organism:

- Size in metric system
- Volume of CNS and Brain
- Numbers of neurons in total CNS and Brain
- Number of celltypes
- Hz for median / average neuron + max. Firing rate.
- Lifespan
- Behavior

C. elegans

	Pros	Cons
Traction (The momentum, existing infrastructure, and potential pathways that make the organism a promising model)	- 10y of development and plausibly by far the easiest organism to achieve full emulation: Simplest model for end-to-end emulation: Cost-efficiently validate emulation approaches against real organism and behavioral data. The scope of all variables is orders of magnitude smaller than in other organisms.	- Limited Extrapolation: Despite being a model organism in neuroscience and medicine (e.g. longevity research), it is hard to extrapolate from the worm to other organisms. Thus its application is limited to very early exploratory research.
Insights (conceptual or theoretical breakthroughs that studying the organism can provide)	- Completeness Threshold: What level of anatomical, molecular, and physiological detail is sufficient to recapitulate worm behavior in silico? Likely the only current model organism where ultra-high resolution models are possible. - Allows to determine the relative importance of different variables to overall emulation success: Specifically, non-synaptic signaling in whole-brain models, gap-junctions, deriving function from structure, extrapolation via celltypes, etc - C elegans promises to be a rosetta stone for converting imaging data into functional models.	- Substantial differences to mammalian nervous systems: Some of the worm's neurons primarily use graded potentials rather than action potentials, which makes direct translation to other organisms challenging (though synapses seem to be more similar to other organisms). - Potentially C. elegans makes particularly heavy use of non-neuronal signaling which may limit the generalizability. - Limited behavioral repertoire: While sophisticated for its size, the behavioral repertoire remains relatively constrained compared to vertebrates. Additionally, the worm lacks clear analogues for many higher cognitive functions of interest (though it has pretty much all the neurotransmitters implied in human cognitive function).

	- Helps to align stakeholders on what constitutes a meaningful emulation to them:	- Little individualism: The opposite side of a highly conserved organism is that it's hard to impossible to study how individual defining characteristics arise.
Operations (The practical and logistical aspects of conducting experiments, collecting data, and executing whole-brain simulations)	- There is substantial infrastructure available already: A decade of open-worm and substantial datasets make it the most matured emulation ecosystem of any organism. - Data acquisition is relatively cheap: The organism is cheaply maintained and can be produced in large numbers. Genetically modifying the organism is possible. No highly organism specialized equipment necessary. Overhead/setup: C. elegans are fairly cheap to maintain; overall setup might be $50,000. - Simulation is computationally easy and can be decentralized: Memory and compute requirements are easily covered by modern consumer hardware. Everyone can participate. - Organisms are highly stereotyped: Accordingly data can be collected from multiple individuals without getting too much interindividual differences	- deformable body and brain making tracking for calcium imaging more difficult

(Anatomy) *Caenorhabditis elegans (C. elegans)* is a worm the size of a grain of salt (~1mm in length, 50 μm in diameter), or 0.002 mm^3. Hermaphrodite worms have a total of 302 neurons

across their bodies, with neurons distributed between a dense anterior "brain" region containing roughly half, and the remainder organized in ganglia and as motor neurons throughout the body (Arnatkevičiūtė et al., 2018). Not all these neurons spike: for example, the 113 motor neurons do not fire action potentials and instead use graded signals since they lack voltage gated sodium channels (Goodman et al., 1998). The typical worm lives for about 15 days, while the longest-living individuals live for approximately 27 days (Mack et al., 2018).

(Behaviors) *C. elegans* exhibits a diverse and adaptive behavioral repertoire, enabling it to thrive in dynamic environments. Its locomotion includes forward crawling, reversals, head movements, and pauses, which combine into higher-order states like "roaming" (rapid movement with few turns) and "dwelling" (slower movement with frequent turns). When food is removed, the worm executes a precise search strategy, starting with local exploration through sharp turns before transitioning to wider-ranging movement. It navigates chemical gradients to locate attractive substances like NaCl and temperature gradients to find its preferred thermal conditions. Remarkably, *C. elegans* can learn from experience, associating specific cues with food or harm. For example, it increases attraction to odors linked to food and avoids chemicals or temperatures associated with negative experiences. Males display additional behaviors, such as leaving food to search for mates, highlighting the worm's behavioral flexibility (Sterling and Laughlin, 2015; Bainbridge et al., 2023).

Videos of C. elegans behavior

C. elegans responding to a touch stimulus

Beyond wakeful behaviors, *C. elegans* exhibits sleep-like states, including developmentally timed sleep during larval molting and quiescence in response to stress or starvation. These states involve periods of reduced movement and responsiveness, akin to sleep in other animals. The worm also displays brief spontaneous pauses during feeding and prolonged quiescence after satiation, mirroring behavioral sequences seen in higher organisms. Together, these behaviors—ranging from basic locomotion and navigation to learning, memory, and sleep—underscore the remarkable adaptability of *C. elegans* and its ability to respond effectively to environmental challenges (Flavell et al., 2020).

Larval Zebrafish

	Pros	Cons
Traction (The momentum, existing infrastructure, and potential pathways that make the organism a promising model)	- **Strong & Growing Community:** Active consortia, open resources (atlases, transgenic lines), imminent first vertebrate connectome release. - **Feasibility–Complexity Sweet Spot:** ~100k neurons with vertebrate-like circuitry yet still small enough for near-complete connectomics and whole-brain imaging. - **Major Potential as Stepping Stone:** Success in fully modeling a vertebrate brain could validate whole-brain emulation broadly and attract large-scale investment.	- **Limited Direct Application:** Larval zebrafish have minimal clinical/industrial utility; overshadowed by mammalian-focused funding.
Insights (conceptual or theoretical breakthroughs that studying the	- **Closer to Mammalian Physiology:** Spiking neurons, layered structures, plasticity—offering richer testbed than C. elegans. - **High-Resolution Structure–Function Mapping:**	- **Larval Constraints:** Optical transparency and tractability largely end after early stages, limiting adult-level complexity and behavior. - **Ecological Validity Gaps:** 3D naturalistic behaviors (e.g., full hunting, social interaction) are hard to record at high spatiotemporal

organism can provide)	Near-whole-brain calcium/voltage imaging can be paired with EM or ExM for synaptic-level detail, enabling correlational and causal studies. - **Diverse Behaviors Even in Larvae:** Prey capture, startle, associative learning, more advanced than many invertebrates, facilitating sophisticated modeling. - **Developmental Window:** Rapid larval development could allow dynamic tracking of circuit formation, potentially illuminating how structural and molecular features shape function.	resolution, raising questions about real-world relevance. - **Unclear Required Detail:** Like other organisms, uncertainty about required molecular detail for accurate simulation, amplified by current lack of though connectome-constrained models.
Operations (The practical and logistical aspects of conducting experiments, collecting data, and executing whole-brain simulations)	- **Manageable Scale & Cost:** Smaller facility/outlay than rodents, feasible HPC demands for ~100k neurons, and simpler housing. - **Whole-Brain Perturbation Feasibility:** Structure-informed whole-brain optogenetics with full-brain imaging is realistic, potentially enabling systematic "perturbation atlases." - **Direct Structural-Functional Integration:** EM or ExM can be matched to in vivo imaging in the very same animals	- **Time-Limited Transparency:** Beyond ~7-14 days, reduced optical clarity and morphological changes hinder long-term/adult study (though special lines can maintain transparency). - **Molecular Tool Gaps:** Rapid larval development complicates viral barcoding or extended protein-expression protocols; genetic toolkit for barcoding relatively underdeveloped compared to other model organisms. - **No Standardized Pipeline:** Fragmentation in data formats, no universal protocol for connecting imaging + connectomics + behavioral data from the same specimen. - **Current Proofreading Bottleneck:** While first EM connectome completion expected within 1-2 years, manual validation currently limits availability.

(Anatomy) The larval zebrafish (*Danio rerio*), defined as the developmental stage preceding 30 days post-fertilization (dpf), possesses a transparent body ranging from 3.5 to 7.8 mm in length

at day 5 (Kimmel et al., 1995; Svara et al., 2022), roughly 500x the size of C. elegans. The larval brain, excluding its spine, measures roughly 0.08mm^3 (400 × 800 × 250 μm, W × L × H) at this stage, contains approximately 100,000 neurons, a number that increases by two orders of magnitude to 10 million in adults (that is, 90-day old or older individuals), whose brains measure 0.4–2 mm in thickness and 4.5 mm in length (Wullimann et al., 1996; Bruzzone et al., 2021; Hill et al., 2003; Hinsch and Zupanc, 2007). These neurons utilize action potential-based signaling – similar to mammals, enabling the study of vertebrate-like neural dynamics. Different neuron types exhibit distinct firing patterns: lateral line sensory neurons operate at frequencies estimated to be up to 80 Hz (Levi et al., 2015) and – early in development – primary motoneurons show rhythmic bursting at 40-50 Hz (Saint-Amant and Drapeau, 2000). The transparency of the larval brain, particularly in pigmentless mutants, facilitates high-resolution optical imaging of neural activity across the entire brain (White et al., 2008; Antinucci and Hindges, 2016). Additionally, larval zebrafish respire through their skin until approximately day 15, allowing for unparalyzed and unanesthetized imaging in agarose-embedded preparations. This feature, combined with the ability to survive for extended periods without external food sources, enables long-duration in vivo neural recordings with minimal maintenance (Hasani et al., 2023). These anatomical and developmental traits make the larval zebrafish a powerful model for studying neural dynamics, structural connectomics, and structure-function relationships in vertebrate neural circuits.

(Behaviors) Larval zebrafish display a diverse and adaptive behavioral repertoire, enabling them to navigate and thrive in dynamic environments (ref). After day 3-4, they exhibit a range of stereotyped movements, including slow swims (scoots), rapid escape responses (C-starts), and specialized maneuvers like J-turns for predation.

Videos of Larval Zebrafish Behavior

Spontaneous movement

Phototaxis, Hunting, Swimming

Current Biology

Source.

These behaviors are finely tuned to sensory inputs: larvae respond to visual stimuli with optomotor and optokinetic reflexes, to acoustic/vibrational cues with escape movements, and to tactile stimuli with highly directional turns. They also display adaptive behaviors such as phototaxis, visual background adaptation, and alarm responses to chemical cues, which help them navigate, camouflage, and avoid predators. Social behaviors, such as shoaling and aggregation, emerge by day 9–10 and are influenced by learned preferences for conspecifics. Additionally, larvae exhibit circadian rhythms, with periods of activity during the day and immobility at night, resembling sleep-like states (Fero et al., 2012).

Zebrafish larvae are also capable of sophisticated forms of learning and memory. They habituate to repeated stimuli, showing distinct forms of rapid, short-term, and long-term habituation that depend on specific neural mechanisms. For example, long-term habituation of the C-start escape response requires protein synthesis and shares mechanistic similarities with learning in other species. Zebrafish larvae can also undergo associative learning - for instance, they learn to move their tails in response to light signals and to avoid areas associated with electric shock. They also display social learning, as shown by how they develop specific preferences for shoaling partners based on their early-life exposure to other fish's appearances (Roberts et al., 2013).

Drosophila

	Pros	Cons
Traction (The momentum, existing infrastructure, and potential pathways that make the organism a	- **Mature Research Ecosystem:** Extensive genetic toolkit, completely reconstructed connectome, rich modeling history, and large active research community spanning decades of foundational work - **Multiple Technical Paths Forward**: Complementary advances in EM, ExM, and X-ray microscopy	- **Limited Direct Application:** Minor clinical/industrial utility; overshadowed by mammalian-focused funding. - **Fragmented Approaches:** No one has done functional imaging and connectomics on the same animal.

promising model)	provide diverse routes to molecular and structural mapping - **Major Potential as Stepping Stone:** Success in modeling the fruit fly's brain could validate whole-brain emulation broadly and attract large-scale investment.	
Insights (conceptual or theoretical breakthroughs that studying the organism can provide)	- **Rich Adult Behavioral Repertoire:** Complex social behaviors, learning, and motor sequences provide sophisticated validation targets. Out-of-distribution behavior not captured by data testable. - **Structure-Function Bridge:** Could illuminate approaches to inferring causal models in systems where exhaustive recording/perturbation isn't feasible	- **Evolutionary Distance:** Further from mammalian architecture than other similarly tractable models like larval zebrafish. - **Unclear Required Detail:** Like other organisms, uncertainty about required molecular detail for accurate simulation.
Operations (The practical and logistical aspects of conducting experiments, collecting data, and executing whole-brain simulations)	- **Theoretical Imaging Access:** Semi-transparency could potentially support whole-brain recording/perturbation at single-neuron resolution. - **Stable Adult Platform:** Unlike larval models, allows extended experiments and complex behavioral studies - **Practical Advantages:** Low-cost maintenance and manageable computational scale (~140k neurons) - **Feasibility–Complexity Sweet Spot:** ~140k neuron, still small enough for whole-brain	- **Tracheal Imaging Barrier:** Air-filled respiratory system creates some challenges for deep-tissue optical access. - **Behavioral Recording Constraints:** Current imaging setups (head-fixation, etc.) limit observable behavioral repertoire - **Validation Framework Gaps:** No clear consensus on success criteria for whole-brain simulations

	connectomics and near-whole-brain imaging.	

(Anatomy) The development of Drosophila melanogaster proceeds through four stages: egg, larva, pupa, and adult. The larval stage represents the primary growth period, with a ~200-fold increase in body mass (Church and Robertson, 1966) and brain expansion from approximately 10,000 neurons (Jiao et al., 2021) to roughly 140,000 neurons forming about 50 million synapses in adults (Dorkenwald et al., 2024). Adult flies at day 7-15 measure 2.5-3 mm in body length and approximately 2 mm in width, with males slightly smaller than females. Under laboratory conditions at 25°C, their mean lifespan typically ranges from 25 to 75 days for females and 25 to 85 days for males, though this can vary significantly depending on factors like genetics and environment (Lints et al., 1983). The adult nervous system comprises two main parts: the brain and the ventral nerve cord. The brain, occupying a volume of about 0.08 mm^3 (Zheng et al., 2018), includes the central brain and paired optic lobes. The ventral nerve cord, located in the thorax, contains approximately 15,000 neurons forming roughly 45 million synapses, and integrates sensory information from peripheral areas while controlling motor outputs (Azevedo et al., 2024). Different neuron types exhibit diverse firing patterns, from circadian pacemaker neurons firing at 1-2 Hz to head direction neurons operating at over 200 Hz during normal behavior (Sheeba et al., 2008; Pisokas et al., 2019). All neural structures are enclosed by the cuticle – a multilayered exoskeleton partially transparent to visible light and transparent to infrared wavelengths (Hsu et al., 2018) – and are oxygenated by the tracheal system, a network of air-filled tubes that branch progressively finer until directly contacting brain tissue.

(Behavior) Drosophila melanogaster displays a wide range of behaviors (Kohsaka., 2023; Caldwell et al. 2003). As larvae, they show movements like crawling forward and backward, sweep their head, or roll over (Kohsaka., 2023; Clark et al., 2018; Caldwell et al. 2003). Sensory feedback modulates these movements, allowing larvae to respond to light, physical obstacles, and food availability (Clark et al., 2018; Busto et al., 1999). Adult drosophila engage in a wide variety of behaviors, including walking, running, grooming, aggression, mating and flying. Over 100 distinct behavioral states have been defined by automated postural analysis (Berman, 2014), with undoubtedly more to be defined in social encounters or in the natural environment. Adult flies exhibit a substantially more complex range of movements than either

C. elegans of Larval Zebrafish, such as rapid banked turns (body saccades) during flight, which help minimize motion blur and avoid collisions (Mujires et al., 2015). Additionally, Drosophila engages in precise grooming sequences, cleaning the head and thorax first before shifting focus to posterior regions, with environmental factors (e.g., temperature and irritation) influencing the order and frequency of these actions (Mueller et al., 2019; Mueller et al., 2020).

Videos of Fruitfly Behavior

Fighting

Male fruit fly courting a female fruit fly

Social mating behavior

Drosophila demonstrates notable capabilities for learning, memory, and communication. It can habituate to repeatedly presented stimuli (a form of non-associative learning), form aversive courtship memories after rejection, and recall outcomes of past aggressive encounters (Durrieu et al., 2020; Zhao et al., 2018; Yurkovic et al., 2006, Youtube Video). Social learning also appears in mate choice, foraging decisions, and predator avoidance, with individuals copying behaviors observed in their peers (Kacsoh et al., 2018; Battesti et al., 2012).

Social behavior is another major facet of Drosophila's ethology and includes aggression, territory defense, and group formation (Monyak et al., 2021; Schretter et al., 2020). Both males and females exhibit aggression, although intensity and tactics differ based on sex and experience - males typically engage in higher-intensity boxing and fencing, while females display more subtle aggressive behaviors (Zwarts et al., 2012, Youtube video). In some species, territoriality is more pronounced, with males defending leaves or fruit patches, whereas in Drosophila melanogaster, territorial claims tend to be conditional and triggered by factors like female presence or food resources (Zwarts et al., 2012; White et al., 2015). Flies also gather on food sources in aggregations that may improve foraging efficiency and promote social information sharing (Philippe et al., 2016), while maintaining individual spacing that adjusts with density and social context (McNeil et al., 2015). Courtship in Drosophila involves an intricate sequence of behaviors that showcase their social communication capabilities. Males perform elaborate courtship rituals including following, wing vibration to produce species-specific songs, tapping, and attempted copulation (Pavlou et al., 2013). The courtship song, produced by precisely controlled wing vibrations, contains specific patterns of pulses and sine waves that are crucial for species recognition and female choice. These acoustic signals work in concert with chemical cues - males detect female pheromones through specialized receptors, while females evaluate male quality through both acoustic and chemical signals (Fernandez et al., 2013, Youtube video). The successful integration of these multimodal cues - acoustic, chemical, and behavioral - determines mating success and helps maintain species barriers (Dweck et al., 2015).

Finally, internal states and social context further influence these behaviors. Isolation can heighten aggression, disrupt sleep – which typically ranges from 6-8 hours during nighttime in wild-type flies (Harbison et al., 2017) – and change locomotor activity, suggesting that Drosophila depends on social cues for regulating stress and energy balance (Eddison., 2021; Li

et al., 2021). After mating, females temporarily become non-receptive and increase egg production, a shift mediated by seminal fluid proteins (Mackay et al., 2005). Temperature preferences also depend on feeding status—hungry flies often choose cooler temperatures, while sated flies gravitate toward warmth (Umezaki et al., 2024). Crowding triggers further behavioral changes, with flies drawing on a sense of "group size" to modify spacing, movement, and interactions (Rooke et al., 2020). These findings highlight how Drosophila's behavioral repertoire is highly plastic, shaped by physiological, environmental, and social factors.

Mouse

	Pros	Cons
Traction (The momentum, existing infrastructure, and potential pathways that make the organism a promising model)	- **Most commonly used model organism in medicine:** Accordingly vast experience with the mouse as a model organism - **Connectome Evaluation running:** BRAINS CONNECTS is evaluating the feasibility of reconstructing a whole mouse brain	-
Insights (conceptual or theoretical breakthroughs that studying the organism can provide)	- **Mammalian brain:** Structure and activity pattern very close to humans. - **Structure to function:** Whole brain recording will be a challenge, thus structure to function mechanisms likely ought to be found. - **Rich social and cognitive behavior:** Verification whether simulations are capable of achieving complex social and cognitive behavior patterns.	-

	- **Critical milestone:** achieving brain emulation in mice is likely the milestone that will trigger massive investments in human scale emulation efforts. - **Various applications in biomedical research**: Given the mouse' role in biomedical research, in-silico simulations might replace some in-vivo experiments.	
Operations (The practical and logistical aspects of conducting experiments, collecting data, and executing whole-brain simulations)	- **Scale Stretch goal:** With large scale investments current technology could suffice to collect sufficient data for brain emulation in mice.	- **Large investments:** The mouse will require investments in the $100M+ range - **Centralization**: The scale of the mouse connectome likely necessitates moving away from a decentralized academic model towards a central large scale facility. This might reduce the numbers of actors that can contribute. - **Whole brain imaging**: It seems unlikely that single-neuron whole brain imaging will be possible for all neurons.

(Anatomy) The house mouse (Mus musculus) is one of the most widely used model organisms in neuroscience. At birth, the typical mouse weighs approximately 1 gram, and males reach 36.3 ± 3.4 grams and females 26.9 ± 3.4 grams (JAX). In laboratory conditions, mice typically live 2-3 years, with some individuals reaching up to 5 years under optimal conditions, though wild specimens rarely survive beyond 1-2 years due to predation and environmental pressures.

The mouse brain reaches approximately 90% of its adult size by two weeks of age (Orr et al., 2016). Neuron counts increase from 57 million at 4 weeks to a peak of 69 million by 15 weeks before stabilizing at 63-70 million in adulthood (Fu et al., 2012, Herculano-Houzel et al., 2006). These neurons are packed into a volume of 420-460 mm^3 (Vincent et al., 2010) – ~5,000x larger than the brains of Drosophila and larval zebrafish (~0.08 mm^3), and roughly 3,000x smaller than the human brain (1,200 cm^3). The neurons are distributed across major regions including the cerebellum (~52 million neurons), neocortex (~8.6 million neurons), and

olfactory bulb (~7.2 million neurons) (Fu et al., 2012). Outside the brain, the spinal cord contains an estimated 8 million neurons (Fu et al., 2012). Mouse neurons exhibit a range of firing rates spanning at least two orders of magnitude in frequency. While the median maximum firing rate across all neurons is 25 Hz, fast-spiking inhibitory neurons can achieve sustained firing rates exceeding 200 Hz (Jimenez, 2020).

(Behavior) . Even before birth, maternal environment and hormonal factors begin molding behavioral tendencies. Throughout development, individual differences in traits like anxiety and exploration emerge and gradually stabilize, though major life events such as social stress or environmental challenges can still significantly influence the adult behavioral phenotype. (Brust et al., 2015)

Videos of mouse behavior

Various complex motor and learning behaviors

Adult mice exhibit a sophisticated behavioral repertoire encompassing complex social, cognitive, and reproductive domains. Their social organization features intricate dominance hierarchies maintained through scent marking, aggressive displays, and both vocal and chemical communication. Cognitively, they demonstrate remarkable capabilities in spatial navigation, associative learning, and behavioral flexibility, readily adapting to environmental changes and remembering both positive and aversive experiences. Even among genetically identical individuals, mice show stable personality differences in traits like anxiety, exploration, and sociability - variations that persist despite standardized laboratory conditions. Their reproductive behavior involves elaborate courtship rituals, and females display comprehensive maternal care including nest building, pup retrieval, and nursing. Throughout adulthood,

these core behavioral patterns remain relatively stable, though aging gradually diminishes exploratory drive, learning speed, and overall activity levels. (Brust et al., 2015)

Miscellaneous Organisms

A variety of other model organisms exist in Neuroscience ranging from other fish like Danionella (ref), over bees, cockroaches, to non-human primates.

The rat brain grows substantially postnatally (Bandeira, 2009) and contains approximately 200 million neurons (Herculano-Houzel and Lent, 2005) distributed across a volume of 1700-2000 mm^3 (Welniak-Kaminska et al., 2019). Typical body is 264.3 ± 87.5 gm and about 398.5 ± 45.1 mm (about 50% body 50% tail). They show highly complex motor, cognitive and social behavior (Videos: ref, this).

The brains of primates represent another significant jump in scale. The common marmoset represents a 15x increase from the mouse brain, with approximately 636 million neurons (Herculano-Houzel et al., 2007) in a brain volume of roughly 6.7 cm3 (Seki et al., 2017). The rhesus macaque brain is roughly another order of magnitude larger, with a brain volume of around 110-130 cm3 (Dash et al., 2023) and a neuron count of approximately 6.4 billion (Herculano-Houzel et al., 2007).

Humans

(Anatomy) The human brain reaches its peak volume in the third to fourth decade of life, typically measuring 1000-1400 cm^3 and weighing approximately 1.3-1.4 kg, or roughly 2% of total body weight. The adult brain measures approximately 140 mm in width, 167 mm in length, and 93 mm in height, though there is considerable individual variation. The brain contains an estimated 86 billion neurons, with distinct regional distributions: approximately 16 billion neurons in the cerebral cortex, 69 billion in the cerebellum (which comprises only 10% of brain volume), and less than 1 billion distributed throughout other brain regions (Azevedo et al., 2009). While estimates for total brain synapses range between 100-1000 trillion, the most precise counts exist for the neocortex, containing an estimated 164 trillion synapses ±17% (Tang et al., 2001). Neural firing rates span several orders of magnitude across

different cell types, with fast-spiking interneurons capable of sustained average firing frequencies exceeding 300 Hz (Wang et al., 2016).

Neurodynamics: Brain Function / Activity

Chapter Summary (AI generated)

Background:

- Neural activity operates across nine orders of magnitude temporally, from millisecond-scale electrical firing to decades of structural changes

- Information processing in the brain involves multiple mechanisms: fast synaptic inputs through chemical synapses, electrical coupling via gap junctions, and slower neuromodulators including neuropeptides
- Understanding brain function requires capturing this complexity across different spatial and temporal scales, which has historically been a significant technical challenge
- Different recording methods offer distinct trade-offs between spatial resolution, temporal precision, and coverage of brain tissue

Advances:

- Modern recording technologies now span from non-invasive methods (fMRI, EEG) that measure aggregate activity of thousands to millions of neurons, to invasive approaches achieving single-neuron precision
- Technical breakthroughs have enabled simultaneous recording of up to one million neurons in select model organisms like larval zebrafish and mice, though typically with trade-offs in temporal resolution or movement constraints
- Development of new tools like GPCR-based fluorescent biosensors has expanded our ability to monitor neuromodulator and neuropeptide signaling in real-time
- Multi-modal approaches combining different recording techniques (e.g., calcium imaging with electrophysiology) are providing more comprehensive views of neural activity
- International collaborations, like the International Brain Laboratory, are successfully integrating data across multiple recording modalities and research sites
- Advances in microscopy techniques, particularly three-photon approaches, are pushing the boundaries of imaging depth and resolution in living tissue

Outlook:

- Technical constraints currently limit whole-brain single-neuron recordings to organisms with brain depths under 1-2mm, making comprehensive recording in larger brains like humans currently infeasible
- Development of standardized, multi-modal recording approaches that combine electrical, chemical, and structural measurements across scales will be crucial for future progress
- Managing and analyzing petabyte-scale datasets from million-neuron recordings presents significant computational challenges that require new analytical approaches
- While recording technology continues to advance, successful interpretation of neural activity will require integration across multiple scales and modalities
- Future progress will depend on both technical innovations in recording methods and development of new computational tools for analyzing large-scale neural data

- The field is moving toward a more complete understanding of how different signaling mechanisms, from fast electrical activity to slower neuromodulatory processes, work together to generate behavior

In the 1920s English physiologist Edgar Adrian wondered if it might be possible to record the electrical activity of a single neuron as it fired, an idea that seemed almost impossible given the technology of the time. Most scientists believed neural signals were too fast and too small to measure. Using a primitive amplifier and an electrode thinner than a human hair, Adrian managed to record the electrical pulses from a single sensory nerve fiber. His 1928 paper "The Basis of Sensation" revealed for the first time that neurons communicate through discrete electrical impulses, or action potentials, and that the frequency of these impulses encoded the intensity of the stimulus. This discovery earned Adrian the Nobel Prize and launched the field of neurodynamics.

Close to a century and many Nobel Prizes later, we now appreciate that neuronal activity is primarily shaped by fast synaptic inputs through chemical synapses and electrical coupling via gap junctions. These signals are modulated by slower-acting neuromodulators, hormones and to some extent by surrounding glial cells that regulate the extracellular environment. The structural features of neurons, particularly their dendritic / synaptic architecture, membrane properties and ion channels, influence how these signals are integrated. Activity-dependent plasticity mechanisms allow neurons to adjust their properties based on past experience. Note that the brain operates over time scales covering about nine orders of magnitude; electrical activity happens at the millisecond timescale, whereas the brain changes *physically* over decades.

Methods of Recording the Brain

The upcoming chapters will differentiate between non-invasive methods such as fMRI, EEG or MEG and invasive methods such as calcium imaging or multi electrode arrays (MEA). Non-invasive methods tend to have a broader penetration depth, i.e. recording across deeper areas of the brain and can record over longer time horizons, whereas they lack spatial resolution and only provide aggregate (indirect) information of the neuronal activity of whole neuronal networks. Invasive methods on the other hand shine by providing spatial resolution of

individual neurons and extremely high sampling rates, i.e. recordings at the millisecond level. Since the first single-cell recordings in the 1950s, these methods have evolved into high-density microelectrode arrays (MEAs) and calcium imaging systems that measure electrical activity of individual neurons. These methods regularly not only require surgery with direct access to the brain, but also often genetic engineering to express fluorescent proteins. The following figure provides an overview of the progress of single-neuron recording over time.

Figures recording speed:

Invasive recording: The number of neurons (log scale) that can be simultaneously recorded is increasing exponentially or super-exponentially. Number of neurons in organisms of interest are indicated for comparison, but don't indicate that whole-brain imaging of those has been achieved or is feasible. (Urai et al., 2022, CC-BY license). Ephys = electrophysiological imaging like MEA and patch clamping, imaging includes modalities such as calcium imaging. Functional imaging the number of neurons being simultaneously recorded has roughly doubled every 5.2 years (Stevenson & Kording, 2011, Urai et al., 2022, Mineault et al., 2024), but has rapidly accelerated since with using light instead of electrodes to extract neuron activity data. Parallel recording of individual neurons over time is now possible for up to one million cells simultaneously.

The combination recording techniques with simultaneous perturbation has been particularly powerful, enabling researchers to observe how manipulating one part of a circuit affects activity throughout the brain. By selectively activating or inhibiting specific neurons, researchers can directly test hypotheses about how neural activity drives behavior. While early studies focused on manipulating broad neural populations, modern approaches aim for increasingly precise control - from targeting anatomically-defined regions to manipulating individual neurons. In the context of simulation efforts perturbation data allows for more targeted collection of less correlated data. The development of more sophisticated perturbation

tools continues to be an active area of research, with particular emphasis on improving spatial precision, temporal control, and compatibility with recording techniques.

While electric coupling via neurotransmitters seem to be the primary way of communication for neurons, neuropeptides modulate neuronal activity and regulate long-term physiological processes, such as structural changes. The scale of signaling peptides is far from millions or billions of neurons, likely hundreds of neuropeptides exist that could interact with their environment in different permutations. This adds a completely new dimension to what needs to be captured to understand information processing in the brain. The totality of neuropeptides interacting with each other is sometimes referred to as "chemical / peptidergic connectome" on top of the electric connectome, which is highly conserved across evolution. (Jekley & Yuste 2024). Unlike classical neurotransmitters, neuropeptides lack specific clearance mechanisms, allowing for sustained signaling that matches behaviorally-relevant timescales (Guillaumin and Burdakov, 2021).

At this point it is not well understood what the relative contributions of neuropeptides to electrical activity and to overall information processing of the brain are. In C. elegans for instance, where the effects of neuropeptides have been studied quite extensively (Bhat et al., 2021), a long list of behavioral defects can be demonstrated in mutants without the respective neuropeptide. The Tachykinin system in Drosophila regulates multiple behaviors including feeding, aggression, and sleep (Nassel and Zandawala, 2022). One prominent example for humans is oxytocin, which has been convincingly demonstrated to influence complex bonding behaviors, e.g. in interactions of mothers and their newborn children (Scatliffe et al., 2019).

Comparison of different recording modalities: The following figure juxtaposes state of the art (non) invasive recording methods to compare their relative information theoretical value across the four dimension volume, resolution (individual data points recorded), temporal resolution in Hz and maximum recording duration in seconds over a day. Across methods and axes tradeoffs become apparent. For comparison the theoretical maximum values for whole brain recording at single cell resolution is presented	

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Non-invasive brain recording methods

Functional Magnetic Resonance Imaging (fMRI)

(How it works) fMRIs uses powerful magnets and radio waves to measure changes in blood oxygen levels throughout the brain. By detecting differences in oxygen-rich and oxygen-poor blood, it identifies regions with heightened neuronal activity (BOLD Signal). As neurons become more active, they consume more oxygen, and this increased blood flow to replenish oxygen is what fMRI visualizes to map brain function. (Availability and Costs) Currently thousands of these devices are used globally for research purposes. A state of the art machines can cost over $10M. Operational costs are often quoted at approximately $1000 / hour.

(Temporal resolution, both sampling rate and upper bound of time sampling) These BOLD signals can rise and fall over roughly 10 seconds which reduces the effective sampling rate from 1 to 0.1 Hz. human fMRI is typically on the orders of 10-20h max with sessions of 1h each (ref). fMRI scanner have the technical ability to perform scans every 1-3s.

(Spatial resolution, penetration depth and neuronal level) fMRI is unusual by being able to usually resolve the whole human brain at up to 1mm^3 resolution. A cubic millimeter can include ~50 000 - 100 000 neurons (The MICrONS project's mm^3 of mouse brain had roughly 100,000 neurons, the cubic mm of human brain tissue scanned 16,000) So an fMRI aggregates the activity of 10s of thousands of neurons. Newer state-of-the-art scanners (Boulant et al., 2024) advance substantially below that approaching structural resolutions (but not temporal) of functional Ultrasound (fUS) at ~0.1 mm^3, equivalent to individual cortical layers. At this resolution 100-1000s of neurons are aggregated. These neurons can potentially be quite heterogeneous though, meaning that vastly different cell types might be firing to generate similar voxel-wise responses.

(Data size and storage requirements) With 32 or 64 receiver coils collecting raw k-space data, along with necessary metadata and reconstruction information, a single hour of high-resolution scanning at $1mm^3$ can generate between 500 GB to 1 TB of total data.. Eventually the fMRI generates over 4 million measurement points per brain. When collecting one volume per second, this generates approximately 15 MB per volume or 60 GB of raw image data per hour of continuous scanning.[1] Across data repositories like OpenNeuro, DANDI, and Brain Image Library on the order of 60TB, approximately the equivalent of 1,000 h of fMRI data, are publicly accessible.

(Data post-processing and quality control) Key to good fMRI pictures are compliant participants. Motion correction and headcases compensate for subtle head movements during scanning, as even millimeter-scale shifts can contaminate the signal. Even with compliant, non-drowsy subjects fMRI data requires extensive processing and quality control before analysis. Temporal filtering removes unwanted frequency components like respiratory and cardiac cycles, while spatial smoothing helps improve signal-to-noise ratio at the cost of some spatial precision. Slice timing correction accounts for the fact that different brain slices are acquired at slightly different times within each volume. Spatial normalization maps individual brains to a standard template, enabling group-level analyses and comparisons across subjects. Modern pipelines (cite) automate many of these steps but require careful parameter selection and validation.

(Behavior that can be correlated, limitations) fMRI is limited in that it is restricted to analysis of supine, generally non-moving participants, meaning that movement and the complete behavior repertoire of organisms is challenging to study. The most commonly studied behaviors include auditory and visual stimuli. To put the BOLD signal in context here: "For naturally spoken English (over 2 words per second), this means that each brain image can be

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[1] (For a $1mm^3$ resolution volume:
Brain dimensions: ~150mm × 180mm × 150mm
This means 150 × 180 × 150 voxels = 4,050,000 voxels
Each voxel requires 4 bytes (32-bit float)
One volume = 4,050,000 × 4 bytes = 16.2 MB
For 1 hour of continuous scanning at 1Hz:
3600 volumes (one per second)
16.2 MB × 3600 = 58.32 GB raw data per hour)

affected by over 20 words". (Tang, 2022). Some of the more sophisticated behaviors include [emotional regulation, moral decision making, finger tapping, etc - add references]

(frontiers in fMRI) Looking ahead, we can expect fMRI to move steadily closer to the resolution mostly provided by invasive methods. Innovations at ultrahigh field strengths, such as the proposed 14 Tesla (the measure of the strength of the magnetic field) human system, promise to deliver sharper spatial resolution (potentially sub-100 μm in specialized regions), improved signal-to-noise ratios, and faster data acquisition. Higher-performance gradients, parallel transmission, dynamic shimming, and novel coil designs—currently being pioneered in the push from 7-11 T to 14 T—could reduce the overall scan time while bolstering temporal precision (Laad, 2023). Methodological improvements in motion-correction and reconstruction algorithms, paired with robust AI-driven pipelines, will help manage the exponential growth in data size.

Electroencephalography (EEG)

EEG records the brain's electrical activity by placing electrodes on the scalp to detect voltage fluctuations produced by large populations of neurons, predominantly cortical pyramidal cells. As neurons synchronize their firing, they generate macroscopic electrical potentials that travel through the skull and scalp. These signals, typically in the microvolt range, must be amplified and digitized before they can be analyzed, reflecting real-time brain activity on a millisecond scale. Because EEG directly measures electrical potentials rather than blood flow or oxygenation, it does not require strong magnets or radiowaves, making it a comparatively simpler and more portable technique. EEG systems are highly accessible, likely with tens of thousands of devices in use worldwide across research labs, hospitals, and increasingly, consumer applications. A research-grade system typically costs between $20,000 to $100,000. Operating costs are relatively modest, mainly involving electrode gel, cap maintenance, and technician time, typically ranging from $50-200 per hour.

The scalp-recorded signals in EEG are blurred by the skull and scalp, which limits spatial precision to roughly one centimeter or more. This resolution aggregates the activity of millions of neurons, similar to fMRI's voxel-level pooling but over broader cortical patches. Unlike fMRI, EEG predominantly captures signals from superficial cortical layers, as signals from

deeper or subcortical structures attenuate significantly before reaching the scalp. Researchers can enhance spatial precision somewhat using high-density electrode arrays (64, 128, or more electrodes) and computational source localization methods, but the fundamental reliance on scalp measurements makes it challenging to pinpoint activity at the resolution of individual cortical layers.

EEG's principal advantage lies in its millisecond temporal resolution, allowing researchers to sample data at rates of 250 Hz to over 1 kHz, i.e. at or above the speed of Neurons. This high sampling rate makes EEG well-suited for capturing rapid neural responses, such as event-related potentials (ERPs) to visual or auditory stimuli. In clinical or research settings, EEG can be collected continuously for hours or even days, enabling extended monitoring of conditions such as epilepsy or multi-night sleep recordings.

Cleaning EEG data is like trying to hear a conversation in a noisy room - you need to filter out various types of interference to get to the brain signals you want. The most common disruptions come from eye movements and blinks (which can be 10-100 times larger than brain signals), muscle activity from jaw clenching or forehead movements, and electrical interference from nearby devices or power lines (showing up as a 50/60 Hz hum in the data). Modern EEG systems handle some of this automatically, using extra sensors near the eyes and sophisticated algorithms to detect and remove these unwanted signals. The data is then typically split into meaningful chunks aligned with experimental events (like when a participant sees a picture or makes a decision), and any segments still contaminated with artifacts are removed. One particularly clever trick is using Independent Component Analysis (ICA) - think of it as having a recording of multiple instruments playing together, and being able to separate out just the violin part. This helps isolate brain activity from various types of noise. After cleaning, researchers can zero in on specific frequencies of interest, from slow delta waves (1-4 Hz) associated with deep sleep to fast gamma oscillations (30+ Hz) linked to active information processing.

Because EEG relies on electrodes attached to the scalp, participants can be seated upright or lying down, and some mobile systems allow moderate movement and study of real-world tasks. Despite this relative flexibility, excessive motion still degrades data quality, and researchers must typically constrain movement more so than with imaging modalities like functional ultrasound

or even fNIRS. The types of behaviors commonly investigated with EEG include perception (visual, auditory), motor tasks (finger tapping, gait), and higher cognitive functions (executive control, working memory). Still, the technique remains limited by relatively coarse spatial resolution, susceptibility to artifacts, and the inability to detect deeper brain structures without invasive methods.

Functional Ultrasound (fUS)

fUS provides great spacial and temporal performance and long time horizons and be something like a continuous partial high resolution fMRI. We have not yet have the chance to look into it more deeply. E.g. Hady et al. (2023) were able to collect fUS data over months with a field of view of around 2x2 cm and a depth of penetration of about 2 cm, a total volume of approximately 8 cm^3.

If you wanted to translate from MEA / Calcium / Voltage imaging to BOLD and use this in humans, fUS would be a really helpful tool.

Miscellaneous non-invasive methods

A variety of other non-invasive brain recording methods exist, but have not been in scope for this report. For comprehensiveness we want to at least mention them here:

- MEG measures the magnetic fields produced by neural activity, offering both excellent temporal resolution (similar to EEG at millisecond precision) and improved spatial resolution (around 2-3mm) compared to EEG, though it requires a magnetically shielded room and supercooling with liquid helium, making it considerably more expensive and less portable than other methods. MEG struggles to reliably measure activity from deep brain structures.
- fNIRS, on the other hand, works similarly to fMRI by measuring blood oxygenation changes but uses near-infrared light instead of magnetic fields, with light-emitting optodes placed on the scalp measuring how much light is absorbed by hemoglobin in the brain tissue beneath; while its temporal (around 1-2 seconds) and spatial resolution (about 1-2 cm) are more modest than fMRI, it offers distinct advantages in being portable, relatively inexpensive, and tolerant of participant movement, making it

particularly valuable for studying infants, allowing naturalistic behaviors, and enabling research in more realistic environments outside the laboratory. fNIRS can effectively measure the outer cortex but cannot access deeper brain structures.

Non-invasive perturbation

Techniques like transcranial magnetic stimulation (TMS) and focused ultrasound (FUS) offer non-invasive methods to modulate brain activity, providing insights into the causal relationships between brain regions and functions.

Invasive optical recording methods

Fluorescence / Calcium Imaging

(How it works) Calcium imaging uses genetically encoded proteins that light up when they bind to calcium - nature's own neural activity signal. These fluorescent proteins, most commonly variants of GCaMP, are introduced into neurons either through viral vectors or transgenic animals. When neurons fire, calcium rushes into the cell, causing these proteins to change shape and become brighter - like microscopic light bulbs turning on. Specialized microscopes, particularly two-photon systems, then capture this fluorescent activity using infrared laser pulses that can penetrate deeper into brain tissue than visible light.

(Preparation and Requirements) The preparation approach varies significantly between viral and transgenic methods. For viral approaches, researchers perform surgery to inject viral vectors carrying the calcium indicator genes and implant a clear window in the skull - a delicate process taking 2-4 hours. After surgery, there's a waiting period of 2-6 weeks for proper protein expression, with expression levels changing over time. Transgenic animals offer more consistent expression across generations but require significant development time. Each approach has distinct trade-offs: viral methods offer flexibility in targeting specific brain regions and cell types but show variable expression, while transgenic lines provide reliable, heritable expression but require months to years to develop and validate.

The technique requires substantial infrastructure - a typical two-photon microscope setup costs between $200,000 to $800,000, with cutting-edge systems exceeding $1.5 million. Globally, the number of two-photon microscopes in active use for neuroscience research is likely in the low thousands. Additional costs include surgical supplies, viral vectors ($300-500 per injection), and specialized habituation equipment. The price per hour falls into the range of 20-100$ / hour (ref, ref)

(Temporal resolution, both sampling rate and upper bound of time sampling) Calcium imaging operates in an interesting middle ground between fMRI and electrophysiology. The calcium indicator GCaMP7f, for example, has a rise time of about 50 milliseconds and decay time of 140 milliseconds, while newer variants like GCaMP8f are even faster. Typical two-photon systems can image at 30 Hz for a single plane, though this drops to 2-5 Hz when imaging multiple planes to capture a volume. Recordings can last from hours in acute preparations to months in chronic experiments. The main limiting continuous imaging is a process called photobleaching, i.e. fluorescent molecules (like the calcium indicators used in neural imaging) permanently lose their ability to fluoresce after being exposed to too much light. Cells continuously produce new indicator proteins, thus recovery timelines are between 6-24h depending on exposure.

(Spatial resolution, penetration depth and neuronal level) The spatial resolution of calcium imaging is remarkable - individual neurons and even their dendrites can be resolved at approximately 0.5-1 μm resolution. A typical field of view might be 500x500 μm, containing hundreds of neurons. Standard two-photon microscopes can image up to 500-600 μm deep in the cortex, while three-photon systems can reach 1-1.2 mm. For advanced systems this results in volumes of up to $1mm^3$ that can be imaged at single cell resolution. Within a single field of view, researchers can typically track 50-500 neurons simultaneously, though some large-field-of-view systems can track over 10,000 neurons at once. Each neuron essentially becomes its own data channel, providing unprecedented detail about neural circuit function. One important problem for now is that scaling to recording more neurons also increases the noise due to imaging limits (ref).

(Data size and storage requirements) The data burden of calcium imaging is substantial. A typical two-hour imaging session at 30 Hz can generate 500 GB to 2 TB of raw data, depending on the field of view and resolution. A single lab might generate 10-50 TB of data

per month. The raw data includes both the imaging movies and associated behavioral measurements. Some labs have reported managing petabyte-scale datasets across long-term projects.

(Data post-processing and quality control) Processing calcium imaging data is a complex pipeline that has evolved significantly. Motion correction is crucial - even micrometer-scale brain movement must be corrected. Modern algorithms can handle both rigid brain motion and warping, though tracking the same neurons across days remains challenging. Cell segmentation - identifying individual neurons in the images - was originally done manually but is now largely automated using deep learning approaches. Converting calcium signals to estimated spike times requires sophisticated deconvolution algorithms that account for the indicator's kinetics.

(Behavior that can be correlated, limitations) The technique enables observation of neural activity during specific, controlled behaviors. Example successful applications include:

- [...]

However, significant behavioral limitations exist:

- [...]

(Frontiers)

Primer on Photon Microscopy

Multiphoton microscopy represents one of the most transformative developments in neuroscience imaging, enabling researchers to peer deep into living brain tissue with unprecedented resolution. It is at the very center of optical recording mechanisms. To understand how different multiphoton approaches work and their relative advantages, let's examine their fundamental principles and historical development.

Traditional single-photon (1P) fluorescence microscopy relies on a fundamental quantum mechanical process: when a fluorescent molecule absorbs a photon of the appropriate energy

(typically in the visible light range), an electron is excited from its ground state to a higher energy level. After a brief period (nanoseconds), the electron returns to its ground state, releasing a photon with slightly less energy (and thus longer wavelength) than the excitation photon. This difference between excitation and emission wavelengths, known as the Stokes shift, is what allows us to separate the emitted fluorescence signal from the excitation light using optical filters.

In the context of brain imaging, this process faces significant limitations. When visible light enters brain tissue, it encounters numerous cell membranes, protein structures, and other cellular components that can either absorb the light or change its direction (scattering). Both effects reduce the number of photons that reach the focal point and make it harder to collect the emitted fluorescence. Moreover, because single-photon excitation occurs wherever an excitation photon encounters a fluorescent molecule, out-of-focus fluorescence creates background signal that reduces contrast and spatial resolution. While techniques like confocal microscopy can reduce this out-of-focus fluorescence using a pinhole, they cannot overcome the fundamental depth limitation imposed by tissue scattering, typically restricting imaging to the first 50-100 micrometers of tissue.

Two-photon (2P) microscopy, first demonstrated by Denk and colleagues in 1990, revolutionized deep tissue imaging through an elegant quantum mechanical principle: instead of using one high-energy photon to excite a fluorescent molecule, it uses two lower-energy photons that arrive nearly simultaneously (within about 10^-16 seconds). These lower-energy photons, typically in the infrared range (700-1040nm), can penetrate tissue much more deeply than visible light because they experience less scattering. Critically, the probability of two photons arriving simultaneously is only high at the focal point of the microscope, creating natural optical sectioning and reducing photobleaching and photodamage in surrounding tissue. This allows imaging up to about 500-600 micrometers deep in the cortex.

Three-photon (3P) microscopy extends these principles further by using three even lower-energy photons (typically >1300nm) to achieve excitation. This technique offers several advantages for deep imaging: the longer wavelengths experience even less scattering, and the requirement for three coincident photons provides better background rejection. 3P microscopy can reach depths of 1-1.2 millimeters, accessing structures like the hippocampus in mice that are largely inaccessible to 2P imaging. However, 3P systems require more expensive laser sources and typically operate at slower speeds due to the need for higher pulse energies.

Because two-photon and three-photon microscopy rely on point-scanning, increasing the number of recorded neurons requires distributing the available laser power across more points and reducing the dwell time per neuron to maintain temporal resolution. Analysis of publicly released datasets

demonstrates this relationship clearly - as the number of simultaneously recorded neurons increases from hundreds to thousands, there is a systematic increase in noise levels unless temporal resolution is sacrificed (Rupprecht, 2021). This relationship holds across different imaging configurations and preparations, reflecting the inherent physical limits of current optical recording approaches.

Recent developments have focused on expanding the capabilities of these systems. Adaptive optics can correct for tissue-induced aberrations, improving image quality at depth. Spatiotemporal multiplexing allows simultaneous imaging of multiple planes or regions. The development of red-shifted indicators has extended imaging depth by reducing scattering of both excitation and emission light.
(Grienberger, 2022)

Voltage Imaging

[This methods chapter needs to get written. We included data in the respective organism sections]
voltage imaging faces two major challenges. First, the fluorescence changes associated with voltage fluctuations are much smaller than those from calcium transients, resulting in inherently lower signal-to-noise ratios that are further degraded by light scattering when imaging deeper in tissue. Second, the high illumination intensities required for detecting these small voltage-dependent changes lead to increased photobleaching and phototoxicity, limiting the duration of recording sessions.

Invasive electrical recording methods

Patch Clamp

MEA

Invasive perturbation experiments

Under one of the most common evaluation criteria for brain emulations, the emulation should match the internal dynamics of the target brain, likely at least at the level of neural activity. Meeting this benchmark requires accurately modeling each neuron's input-output

function—how its outputs depend on the various inputs it receives. While passive recordings do provide constraints on possible input-output functions, common inputs and the fact that different circuit configurations can produce identical activity patterns make it challenging to gather enough data to uniquely identify each neuron's parameters (Haspel et al., 2023). This fundamental limitation persists even with extensive recordings under diverse conditions, as the system's inherent complexity and feedback loops mean that many different parameter sets remain consistent with observed activity patterns (Pospisil et al., 2024). This challenge of parameter identification from passive recordings alone - which exists even when the connectome is known - has led to increasing emphasis on interventional methods.

Invasive perturbation methods provide a solution by breaking natural correlations between neurons and allowing direct manipulation of specific circuit elements. As established in causal inference theory, determining genuine causal relationships generally requires perturbations (Woodward, 2005; Pearl, 2009). In neural circuits specifically, establishing the causal influence of one neuron on another requires stimulating the former while recording from the latter (Haspel et al., 2023). By precisely controlling individual neurons or groups of neurons while recording from others, experimenters can drive circuits into novel states that wouldn't arise naturally. Data from such causal perturbations enables dramatically more sample-efficient fitting of neuronal input-output functions compared to passive recordings alone. These targeted interventions thus provide crucial constraints for reverse engineering neural computation.

Patch clamp recording, the oldest of these interventional techniques, provides extremely precise control and measurement of individual neurons' electrical activity. By forming a tight seal with the cell membrane, it enables direct manipulation of membrane potential while simultaneously recording with sufficient temporal resolution to detect individual action potentials. To establish causal relationships between neurons, dual patch recordings are required - stimulating one neuron while recording from another. This method produces the highest-quality data for determining input-output functions, with minimal off-target effects and exceptional signal fidelity. However, patch clamp recordings are technically demanding, labor intensive, and typically limited to short durations. Combined with the need for dual recordings, this makes it impractical for mapping large circuits.

Optogenetics enables precise temporal control of genetically defined neuron populations using light-sensitive ion channels or pumps. This approach allows simultaneous manipulation of many neurons and can be combined with large-scale recording methods. Standard optogenetic approaches using fiber optics suffer from light scattering in tissue, limiting spatial resolution. However, methods like two-photon holography combined with soma-targeted opsins can achieve single-cell resolution in vivo, though spatial precision varies with the specific tools and light delivery systems used. While optogenetic stimulation offers millisecond-precise control, recording neural activity in response to these perturbations presents additional challenges. The most common recording method, calcium imaging, struggles to reliably detect individual spikes, particularly in fast-spiking neurons, despite ongoing improvements in indicators. Furthermore, optical crosstalk between stimulation and imaging wavelengths can interfere with simultaneous recording and perturbation.

Chemogenetics complements these approaches by offering sustained but less temporally precise control through engineered receptors (DREADDs) that respond to specific synthetic compounds. After viral delivery of these receptors to targeted neuron populations, systemic drug administration can modulate neural activity over hours to days. While this approach enables manipulation of specific cell types and even specific neural projections through retrograde viral vectors, its utility for reverse engineering precise input-output functions is limited. The slow kinetics of drug action and intracellular signaling cascades, combined with the sustained nature of the manipulation, make it impossible to probe the kind of precise temporal interactions needed to characterize neuronal computation. Instead, chemogenetics is most valuable for behavioral studies and understanding the role of broader cell populations over longer timescales.

Invasive neuromodulator recording methods

A variety of methods to capture the effects of neuromodulators exists (see figure from Girven et al). For the sake of this report we will focus on the most prominent recording method, G-Protein Coupled Receptor (GPCR) Fluorescent Biosensors.

Figure from Girven et al 2022: Tools for Sensing Neuropeptide Expression, Activity and Transmission. This graph serves to schematically compare contemporary tools used for sensing neuropeptide expression across different spatial and temporal resolutions. [Most of these are exploratory - we are describing the most established ones in separate subchapters] Colors represent a technique's ability to detect endogenous release of neuropeptide (see "Neuropeptide Sensing" scale). Sphere size denotes a technique's ability to detect neuropeptide expression and/or cell activity. Larger spheres denote techniques that are better at either detecting neuropeptide expression in a neural population or neuropeptide-expressing neuron activity. Smaller spheres represent techniques that may be instead manipulating neuropeptide transmission and therefore conclusions are typically drawn from observed behavioral phenotypes. Technique acronyms are as follows: CRISPR – clustered regularly interspaced short palindromic repeats gene editing; SCRNAseq – single cell RNA sequencing; SPTX – Spatial Transcriptomics; merFISH - Multiplexed error-robust fluorescent in situ hybridization; ELISA – enzyme-linked immunosorbent assay; LC-MS – liquid chromatography mass spectrometry; NPRRs – neuropeptide release reporters; Biosensors - Fluorescent GPCR-based biosensors; Ephys - electrophysiology; FSCV – fast scan cyclic voltammetry.

(How it works) GPCR sensors are engineered protein-based tools that enable direct visualization of neuromodulator and neuropeptide signaling in the brain. These sensors are created by inserting a circularly permuted fluorescent protein (cpFP) into the intracellular loop of native GPCRs sitting in cell membranes, effectively coupling them to fluorescent signals. The engineering process blocks endogenous G-protein binding while maintaining the receptor's natural ligand affinity, thus preventing downstream signaling cascades while preserving detection capabilities. Unlike calcium indicators that measure neuronal activity indirectly through calcium influx, GPCR sensors directly detect specific neuromodulator and neuropeptide binding events. (Girven et al., 2022)

Figure from (Muir, 2024)

(Availability and Costs) Like calcium imaging, GPCR sensors can be delivered via viral vectors (typically AAV-based) or through transgenic approaches. However, achieving adequate signal-to-noise ratios often requires higher laser powers and more sensitive detectors, typically increasing setup costs by some extent compared to calcium imaging systems. Current validated sensors exist for approximately 15 different neuromodulators and neuropeptides, including dopamine, norepinephrine, serotonin, acetylcholine, and opioid peptides, with 2-3 different variants typically available for each target (Muir, 2024).

(Temporal resolution, both sampling rate and upper bound of time sampling) While calcium indicators track neural activity with ~100ms precision, GPCR sensors provide direct measurement of neuromodulator binding with comparable temporal resolution. Current variants such as dLight1.3b achieve response times of 9.5ms for dopamine binding and 90ms for unbinding. Recording durations match those of calcium imaging (hours to months), though photobleaching rates can be 2-3 times higher due to increased excitation requirements.

(Spatial resolution, penetration depth and neuronal level) These sensors provide sub-second temporal resolution for detecting ligand binding events. is primarily limited by expression stability and photobleaching, but e. Current technology allows simultaneous monitoring of up to 3 different neuromodulators using spectrally separated sensors (e.g., green dLight1.3b for dopamine alongside red GRABNE2m for norepinephrine) (Muir, 2024). Recording duration and depth limitations match those discussed for calcium imaging: experiments can span from acute recordings (hours) to chronic monitoring over weeks or months at volumes up to approximately 1 mm^3.

(Data size and storage requirements, processing) Similarly, data generation and processing requirements closely parallel those described for calcium imaging, with similar motion correction and signal extraction needs.

(Behavior that can be correlated, limitations) GPCR sensors have enabled several behavioral studies. Specific examples include (Girven et al., 2022, Muir, 2024):

- Tracking dopamine release in nucleus accumbens during reward anticipation vs consumption, revealing distinct temporal patterns (100ms precision) that predict behavioral choice
- Monitoring opioid peptide release in the arcuate nucleus during feeding, demonstrating selective activation during food deprivation but not during normal feeding
- Simultaneous imaging of acetylcholine and norepinephrine release in basal forebrain during sleep-wake transitions, showing sequential activation patterns with 1s precision

(Frontiers in GPCR sensors) From the conclusion section of Muir, 2024: “Looking to the future, multiplexed recording or manipulation of multiple molecules at once may reveal new principles of how neuromodulators and neuropeptides alter circuit plasticity in specific cell types and at dis- tinct subcellular locations. It will be critical to continue engineering sensors

and actuators with excitation and activation spectra that avoid overlap with existing sensors for calcium, voltage, or neurotransmitters [...] Machine learning– and artificial intelligence– based engineering (57, 147) and de novo pro- tein design (148) may enable the creation of completely orthogonal tools that are tuned to only a single analyte and can still maintain genetic encodability and cell type specificity."

Data science: storage, standardization, analysis

Datasets

Functional neuroscience datasets are often shared on dedicated data repositories. Recently, Mineault et al, 2024 published an analysis of the "contents of DANDI, OpenNeuro, iEEG.org, as well as large-scale individual datasets" as part of their NeuroAI for AI Safety roadmap. We will quote them verbatim here:

Availability of neural data to train a large-scale model (from Mineault et al, 2024)
"The past decade has seen an explosion in the quantity of neural data freely available online. These public datasets represent a unique opportunity to learn good representations of neural data for a variety of downstream tasks, including brain-computer interfaces, clinical diagnoses for computational psychiatry and sleep disorders, and basic neuroscience.

Here we present a breakdown of the available data sources from an analysis of the contents of DANDI, OpenNeuro, iEEG.org, as well as large-scale individual datasets. Some of the highlights from this analysis include:

- There are around 100,000 hours of neural data available in freely accessible archives.
- There are roughly 3.3 million neuron-hours of single-neuron recordings from animals.
- The most abundant data type in terms of number of hours is intracortical EEG in humans–an invasive modality generated from the typically continuous, week-long recordings performed during epilepsy monitoring.
- Single neuron data is concentrated in a few datasets; the top 10 largest datasets in terms of neuron-hours account for more than 94% of total neuron-hours across all of DANDI. These come mostly from zebrafish and mouse, with one dataset from macaques.
- Large fMRI recordings are split into two categories: broad neuroimaging surveys, including HCP and UK Biobank, which scan many people for a short time; and intensive

neuroimaging datasets [174], including Courtois Neuromod and the Natural Scenes Dataset, which scan few people for a very long time."

Total recorded hours by recording technology
MEG
Calcium
Ephys
EEG
fMRI
iEEG
138.6 hours
1.4K hours
6.1K hours
14.5K hours
32.2K hours
49.0K hours
1.0
10.0
100.0
1.0K
10.0K
100.0K
total hours

OpenNeuro: fMRI
number of subjects
hours of recorded data per subject
12.5k hours
2.5k hours
500 hours
100 hours
20 hours
cneuromod >>

DANDI: single neuron recordings
number of subjects
hours of recorded data per subject
100
10
1
0
10
20
30
40
50
60
70
1600 hours
800 hours
400 hours
200 hours
100 hours
50 hours
Mouse
Rat
Macaque
Human
Zebrafish
Marmoset
Ephys
Optical

Many additional databases exist, with a list of over 50 available here:

Neurodata repositories Importantly, this best practice of public data sharing has not been the default over the past two decades and is only slowly finding adoption, as some funders make it a requirement. Only recently efforts like DANDI started to standardize neurophysiology data, so they are more comparable and interoperable. From private conversations with multiple experts, they assume that less than 5% of all the data in existence is publicly available (in any form) at this point. In conversations with computational neuroscientists the anecdotally reported access to multiple consistent and well cleaned datasets to be one of the main limiting factors. This data barrier also prevents many excellent engineers from other fields to get involved and make meaningful contributions.

We can roughly think about them as 4 categories.

1. **Preferred repositories:** these comprise the majority of the well-formatted, easily accessible data: OpenNeuro, DANDI, Brain Image Library, EBrains, FigShare, Allen Institute, or Zenodo.
2. **Mediocre repositories:** repositories like CRCNS, Neurovault, and others are outdated and don't have much data that's easily accessible.
3. **Meta-repositories, registries, and misfits:** neuinfo doesn't host data and just has metadata scraped from elsewhere. OpenEphys mostly lists other repositories, and the data it does link might just be repeats of previously mentioned repositories.
4. **Single datasets:** Study Forrest and FlyWire are examples of large datasets hosted on their own website.

While some data repositories offer APIs facilitating mass data download, others may be impossible to scrape due to absent or scattered metadata; despite this, the user-friendliness of a repository correlates positively with data volume, allowing access to significant portions of data across various modalities. Concerns include the potential for duplicate data across repositories—though not currently significant—and data heterogeneity, with image data being particularly vulnerable to quality issues like poor resolution. The size of both repositories and dataset seems quite Pareto-distributed, with the largest ones vastly larger than the median. Therefore we expect to focus primarily on a few key repositories and self-hosted datasets, expanding to others as needed. This also allows us to very roughly estimate total amounts of data based on what we can see, usually within a factor of 2 of what is easy to collect.

Standardization

The standardization of neurodynamic datasets presents unique challenges due to the extraordinary complexity of neural recordings across different temporal and spatial scales across different organisms. These technical challenges are compounded by sometimes significant methodological variations across laboratories and institutions. Different research groups often employ distinct preprocessing pipelines, quality control procedures, and analysis methods, making direct comparisons between datasets difficult. Even within the same recording modality, variations in experimental protocols, equipment configurations, and environmental conditions can introduce systematic differences that complicate data integration. The situation is further complicated by inconsistent documentation practices, with many datasets lacking

crucial metadata about preprocessing steps, quality control measures, or experimental conditions.

This lack of standardization hinders meta-analyses across datasets as well as large scale machine learning approaches. While initiatives like DANDI have begun to address these issues by developing standardized formats and metadata schemas, significant work remains to be done. The successful integration of diverse neuroscience datasets will require not only technical solutions for data formatting and quality control but also broader community agreement on best practices for data collection, preprocessing, and documentation. A promising example of such an effort is the international brain laboratory, an initiative that was able to collect a "comprehensive set of recordings from 115 mice in 11 labs performing a decision-making task with sensory, motor, and cognitive components, obtained with 547 Neuropixels probe insertions covering 267 brain areas in the left forebrain and midbrain and the right hindbrain and cerebellum".

Data analysis methods

The advent of high-density neural recording technologies has transformed neuroscience, routinely generating datasets that were unimaginable a decade ago. Two-photon calcium imaging can now capture the activity of over 100,000 neurons simultaneously at 30Hz, while high-density electrode arrays like Neuropixels can record from thousands of neurons at 30kHz. This scale of recording presents both opportunities and fundamental challenges for data analysis. A single one-hour recording session with calcium imaging can generate approximately 2TB of raw video data, which must be processed and analyzed to extract meaningful insights about neural computation and behavior.

The primary conceptual challenge in analyzing these datasets is not just their size, but understanding what we can learn from them that wasn't possible with smaller recordings. The answers are emerging on multiple fronts: the ability to study coordinated activity across large populations, the identification of rare cell types and response properties, and perhaps most importantly, the capacity to observe computation happening simultaneously across multiple spatial scales in the brain.

Initial Processing Pipeline The journey from raw data to analysis-ready neural signals involves several computationally intensive steps. For calcium imaging, the first challenge is motion correction – compensating for brain movement during recording. Modern GPU-accelerated algorithms can process 512x512 pixel frames at 30Hz in real-time, a crucial capability for closed-loop experiments. Following motion correction, cell identification and segmentation algorithms like Suite2p can process hours of recording in parallel, identifying up to 100,000 neurons in several hours on a standard workstation.

For electrophysiology, the initial bottleneck is spike sorting – the identification of individual neurons from mixed electrical signals. Current algorithms like Kilosort4 can process 384-channel recordings in near real-time with over 90% accuracy, though higher channel counts require proportionally more computational resources. A key advance has been the development of automated quality metrics that can reliably identify well-isolated units without human intervention, making it possible to process the large datasets generated by chronic recordings.

Population Analysis and Computational Requirements The scale of modern recordings has revealed that neural populations often operate in higher-dimensional regimes than previously thought. Early studies suggesting low-dimensional neural dynamics were likely limited by recording only small populations. With large-scale recordings, we now see that both stimulus-evoked and movement-driven population activity in sensory cortex is high-dimensional, following power-law distributions of variance across principal components. This understanding has practical implications for analysis approaches.

Current hardware and algorithms can handle different scales of analysis with clear computational requirements. Real-time processing of 10,000 neurons requires a modern workstation with 32GB RAM and a high-end GPU. Scaling to 100,000 neurons demands 128GB RAM and multiple GPUs, with processing taking 3-5x real-time. At the extreme end, analyzing 1,000,000 neurons requires distributed computing systems with at least 1TB RAM, processing at 10-20x real-time. The primary bottlenecks emerge in population analyses that require pairwise computations between neurons – for instance, a correlation matrix for 100,000 neurons requires 80GB of RAM just for storage.

Scaling Challenges and Future Directions Scaling these analyses to even larger populations (millions to billions of neurons) would require fundamental algorithmic innovations. Current approaches often scale quadratically with neuron count, making them impractical for very large populations. Several promising directions are emerging: hierarchical processing approaches that analyze local neural clusters before global computations, sparse approximation methods that maintain only significant neural interactions, and online processing strategies that avoid storing complete datasets in memory.

The field is also seeing increased focus on more complex and ethological behaviors, particularly in freely moving animals. These scenarios create new analytical challenges due to the large number of potential nuisance variables (environmental factors, spontaneous movements) that are difficult to control. This has driven the development of model-based approaches for iteratively refining and testing hypotheses through manipulations of the environment, task, or neural activity.

Cross-Validation and Statistical Rigor A critical lesson from large-scale analyses has been the importance of rigorous statistical approaches. The high dimensionality of the data makes it easy to find apparent structure that doesn't generalize. This necessitates careful cross-validation procedures for all analyses, from basic tuning curves to complex dynamical models. Temporal correlations in neural data create particular challenges – simple random splitting of time points can create spurious results, requiring instead the splitting of data into extended blocks of time.

Looking forward, success in analyzing ever-larger neural populations will require innovations in both algorithms and computing infrastructure. The field is moving toward a hybrid approach that combines the statistical rigor of traditional analyses with the scalability of modern machine learning methods. This evolution is supported by new training initiatives, such as the Neuromatch Academy, which help experimental neuroscientists develop the computational expertise needed to work with these complex datasets.

From Stringer et al 2024

An abundance of data available for analysis. (**A**) Two-photon calcium imaging from multiple visual cortical areas in a behaving mouse. (**B**) Raster plot of neural activity from the recording in (A), showing coordinated activity across timescales and spatial scales.

Organisms

Neuron counts across the animal kingdom span over ten orders of magnitude (see figure below). Current capabilities for single neuron recording going up to ~1,000,000 Neurons in mice (~1% of the brain), and climbing up to 100 Million neurons is a challenge for the upcoming decade in neuroscience. Beyond, it is possible that physical limitations will prevent single neuron recording (Marblestone, 2013).

[Stand-in] Figure X. The Single Neuron Recording Frontier.. Smaller organisms like C.elegans, Larval Zebrafish or Fruitfly. The frontier for the next decade likely ends with the mouse and then potentially transitions into a territory that might be physically impossible to measure at single neuron resolution.

Approx.
neuron count
10^{12}
Elephant
10^{11}
Human
Chimp
Physically impossible?
10^{10}
Rhesus Macaque
Dog
10^{9}
Guinea pig
10^{8}
Mouse
Frontier next decade
10^{7}
Etruscan shrew
Honey bee
10^{6}
Cockroach
Fruitfly
Crayfish
10^{5}
Zebrafish
Pond Snail
Sea slug
10^{4}
Megaphragma wasp
Current technical capabilities
10^{3}
C. elegans
10^{2}
10^{1}

Despite the impressive progress in recording capabilities, **no single neuron whole brain imaging (>90% of all neurons recorded simultaneously) has been achieved for *any* organism to date**. C. elegans recordings go up to roughly 50% of all neurons, up to 68% in Drosophila and around 80% in Larval Zebrafish. Various reasons why that is the case will be discussed below or have been gestured at in the anatomy sections above.

Notably these numbers come with additional limitations such as temporal resolution substantially below neuronal firing rates, short recording durations and importantly fixation of the animal. The following figure represents an aggregation of all studies that were available to this report. It highlights a couple of trends: smaller organisms have more data available and are more likely to have significant data without fixation. Also recording duration in all organisms is at least 2-3 orders of magnitude away from the actual life span. Finally, recording modalities with strong performance in one, will likely have poor performance in another dimension.

Overview Figure Neurorecording: Radar plots of all model organisms and humans based on the most advanced recordings in the past decade. As shown in figure X above, multiple dimensions need to be kept in mind for neuronal recordings: spatial resolution, brain volume, temporal resolution, and individual / cumulative recording duration. The plots split recordings from fixated (left) and freely moving experiments (right). The outer ring is normalized to the maximum known values in the respective organism. Each ring represents one order of magnitude.

Currently the order in this figure is: ['C. elegans', 'Zebrafish', ‘Drosophila’, ‘Mouse’, 'Human']

Temporal resolution: Hz
Max. Neuron Firing Rate: 1.0 × 10¹ Hz
Avg. Lifetime: 2.2 × 10⁴ min
Duration single session min
Number of neurons
Total Neurons: 3.0 × 10²
Avg. Lifetime: 2.2 × 10⁴ min
Duration total repeated sessions min
Resolution in µm isotropic
Single Neuron Resolution: 1.0 × 10⁰ µm³
Temporal resolution: Hz
Max. Neuron Firing Rate: 1.0 × 10¹ Hz
Avg. Lifetime: 2.2 × 10⁴ min
Duration single session min
Number of neurons
Total Neurons: 3.0 × 10²
Avg. Lifetime: 2.2 × 10⁴ min
Duration total repeated sessions min
Resolution in µm isotropic
Single Neuron Resolution: 1.0 × 10⁰ µm³
Temporal resolution: Hz
Max. Neuron Firing Rate: 2.0 × 10² Hz
Avg. Lifetime: 5.8 × 10⁴ min
Duration single session min
Number of neurons
Total Neurons: 1.4 × 10⁵
Avg. Lifetime: 5.8 × 10⁴ min
Duration total repeated sessions min
Resolution in µm isotropic
Single Neuron Resolution: 1.0 × 10⁰ µm³
Temporal resolution: Hz
Max. Neuron Firing Rate: 8.0 × 10¹ Hz
Avg. Lifetime: 1.4 × 10⁴ min
Duration single session min
Number of neurons
Total Neurons: 1.0 × 10⁵
Avg. Lifetime: 1.4 × 10⁴ min
Duration total repeated sessions min
Resolution in µm isotropic
Single Neuron Resolution: 1.0 × 10⁰ µm³
Temporal resolution: Hz
Max. Neuron Firing Rate: 2.0 × 10² Hz
Avg. Lifetime: 1.6 × 10⁶ min
Duration single session min
Number of neurons
Total Neurons: 7.0 × 10⁷
Temporal resolution: Hz
Max. Neuron Firing Rate: 2.0 × 10² Hz
Avg. Lifetime: 1.6 × 10⁶ min
Duration single session min
Number of neurons
Total Neurons: 7.0 × 10⁷

Heatmap plot of significant brain recording publications across different organisms. The data is the same as in the radar chart, but broken down by the author.
Note that often data was lacking and a best-guess approach was followed as there wasn't yet the time to go through all the publications in detail.

C. elegans

(invasive work) Typical GCaMP-based approach at moderate laser power and short epochs, allow repeated short sessions (5–15 minutes each) over several days totalling in a few hours of imaging across its life of a worm without severely compromising the animal. Proof-of-concept whole-brain or whole-body calcium imaging of C. elegans has been achieved, e.g. using light field microscopy (Prevedel et al., 2014), a fully automated tracking platform to enable freely-moving imaging (Li et al., 2021), improved detection, tracking, and segmentation algorithms (Wu et al., 2022, Lanza et al., 2024) Recent advances in live animal labeling, such as NeuroPAL (Yemini et al., 2021), create unique fluorescent labels of each neuron in the worm, allowing for almost unambiguous identification of each of the 302 neurons in freely behaving worms, without being able to record from them. No large-scale whole-body neuron activity datasets of C. elegans are available as of today (Sprague et al., 2024, Simeon et al., 2024) and voltage indicators have been difficult to successfully implement in C. elegans, so work continues. Atanas et al., 2023 performed imaging of approximately 60 freely moving animals for a total of ~1,000 minutes of imaging, measuring the activity of approximately 150 head neurons (Atanas et al., 2023). Individual sessions per animal were 16 minutes, often split into two 8-minute segments, and temporal resolution 1.7 Hz. They used a nuclear calcium indicator (NLS-GCaMP7f) and a spinning disk confocal for volumetric fluorescence imaging, coupled with brightfield imaging of the worm's behavior. After processing, this generated about 30 GB.

(Voltage, Patch Clamp, MEA) Patch-clamp recordings in C. elegans allow researchers to directly measure electrical activity in neurons and muscles with millisecond precision, making it ideal for studying ion channels and synaptic connections. The main drawbacks are that it requires careful dissection of the worm's protective outer layer and can only record from immobilized animals, making it impossible to study neural activity during behavior (Goodman et al., 2012). Voltage imaging with GEVIs offers a complementary approach, enabling researchers to measure electrical activity across multiple cells simultaneously in living, behaving worms. The main limitation is that GEVIs produce relatively weak fluorescent signals compared to calcium indicators, though recent advances using rhodopsin-derived indicators, such as Arch-based GEVIs, have improved their brightness and sensitivity (Hashemi et al., 2019;). Tokunaga recorded individual neurons from ~100 individuals for 30-60s at 20-250Hz (Tokunaga et al., 2024).

(Neuropeptides) The C. elegans genome encodes over 300 neuropeptides processed from approximately 160 precursor genes, along with around 150 peptide G protein-coupled receptors (GPCRs) (Taylor et al., 2021). Recent large-scale deorphanization efforts have identified 461 peptide-GPCR signaling pairs, providing unprecedented insight into the organization of peptidergic signaling (Beets et al., 2023). This work revealed that while some peptide-GPCR interactions are highly specific, others display complex combinatorial patterns - individual neuropeptides can activate multiple receptors, and single receptors can respond to various peptides (Beets et al., 2023; Ripoll-Sánchez et al., 2023).
Functional studies indicate neuropeptides operate across diverse timescales in C. elegans, from seconds to hours. While typically considered slow modulators, some neuropeptides like NLP-40 can trigger responses within seconds. Recent whole-brain imaging combined with optogenetics has revealed that many rapid functional connections between neurons depend on dense-core vesicle release, suggesting neuropeptides extensively shape fast neural dynamics (Randi et al., 2023). The peptidergic network appears organized into distinct functional modules, including specialized "hub" neurons that are rich in dense-core vesicles and highly connected through peptidergic signaling (Ripoll-Sánchez et al., 2023).

(Perturbation) Due to its anatomy C. elegans represents an exemplary model organism for perturbation studies. Since the first expression of optogenetic proteins in C. elegans in 2005 (Boyden et al., 2005), optogenetic studies have revealed numerous insights into neural circuit function (Piatkevich and Boyden, 2023): neurons controlling locomotor rhythms (Fouad et al., 2018), interneurons that integrate multiple olfactory inputs to represent valence (Dobosiewicz et al., 2019), single neurons that can both encode chemotactic memory (Luo et al., 2014) and regulate multiple behavioral outputs (Li et al., 2014), circuits mediating behavioral state switching in response to oxygen levels (Laurent et al., 2015), and specific interneurons controlling chemotaxis programs (Kocabas et al., 2012). Additionally, optogenetics has revealed how synaptic energy demand regulates glycolytic protein clustering and identified neurons that contribute oscillatory activity controlling backward locomotion (Piatkevich and Boyden, 2023). Recent technical advances have dramatically expanded the scale and precision of perturbation studies in the worm. Sharma et al. developed TWISP (Transgenic Worm for Interrogating Signal Propagation), a strain expressing both optogenetic actuators and calcium indicators in all neurons while avoiding optical crosstalk –unwanted interference between light

signals used for neural activation and activity measurement (Sharma et al., 2023). Moreover, Randi et al. systematically measured signal propagation between 23,433 pairs of neurons in the worm's head through direct optogenetic activation combined with whole-brain calcium imaging, revealing that actual signal flow often differs from predictions based on anatomical connectivity due to extrasynaptic signaling (Randi et al., 2023).

Larval Zebrafish

(invasive) Neural activity in larval zebrafish can be recorded using either head-fixed or freely-moving preparations (Hasani et al., 2023).

In **head-fixed preparations**, the fish's head is immobilized in agarose, which limits natural behavior but enables stable imaging. Several approaches have been developed to increase behavioral output: the tail can be freed to monitor movement intentions, and fish can navigate virtual environments by using their tail movements as input (Ahrens et al., 2012; Trivedi and Bollmann, 2013; Vladimirov et al., 2014; Torigoe et al., 2021). Initial progress in whole-brain imaging came from one-photon techniques like light-sheet microscopy, which achieved simultaneous recording of ~80,000 neurons (about 80-90% of all neurons) at 1 Hz (Ahrens et al., 2013), later improved to 4 Hz using electrically tunable lenses (Favre-Bulle et al., 2018). Recent advances include DaXi microscopy, achieving 3.3 Hz over 0.03 mm^3 (37.7% of total brain volume, approx. 40,000 neurons), and SCAPE microscopy reaching 25.75 Hz over smaller volumes of 0.00481mm^3 (6% total brain volume, approx. 6,000 Neurons) (Yang et al., 2022; Voleti et al., 2019). However, in all one-photon approaches, scattered light from the excitation laser is visible to the fish and can interfere with presented visual stimuli. Two-photon microscopy uses infrared light invisible to the fish, though care must be taken as fish eyes absorb infrared light and exposure can be lethal. While two-photon imaging eliminates visual interference, its main limitation is speed: current technology can record 52,000 neurons distributed throughout 80% of the brain's volume at 1 Hz (Bruzzone et al., 2021). However, even with these advances, head-fixed preparations fundamentally constrain the fish's natural behavior, particularly during complex tasks like hunting.

Recording neural activity in **freely moving zebrafish** presents unique technical challenges, as larvae move in rapid bursts reaching speeds of 10 cm/s and angular velocities of 600°/s, i.e. close to 2 revolutions around themselves per second (Johnson et al., 2020; Mearns et al., 2020).

These movements require sophisticated real-time tracking to keep the fish within the microscope's field of view. Higher imaging speeds typically come at the cost of either reduced spatial resolution or smaller field of view. Current tracking approaches either move the stage to cancel the fish's motion (Kim et al., 2017) or use mirror systems to keep a stationary microscope focused on the fish (Symvoulidis et al., 2017). Several imaging techniques have been developed, including differential illumination focal filtering microscopy (DIFF), light field microscopy (LFM), and hybrid approaches combining LFM with confocal or light-sheet methods (Cong et al., 2017; Zhang et al., 2021). Additional challenges include calcium indicators being too slow to track rapid neural changes during behaviors like hunting, and current setups restricting the fish's vertical movements, which are crucial for natural hunting where fish prefer to strike at prey from below (Bolton et al., 2019; Mearns et al., 2020). Additionally, counteracting the fish's movements by moving the imaging chamber can significantly alter their behavior, reducing how far, fast, and often they move (Kim et al., 2017).

(Voltage, Patch Clamp, MEA) Overall, calcium imaging speeds still falls roughly two orders of magnitude behind the fastest neurons in zebrafish larvae (Anonymous authors, 2024). Voltage imaging in larval zebrafish has also made remarkable strides in recent years, bringing the field closer to the goal of whole-brain voltage imaging at cellular resolution. Innovations in genetically encoded voltage indicators (GEVIs), such as ASAP3-Kv, Voltron2-Kv, and Positron2-Kv, have significantly improved brightness and signal-to-noise ratio, enabling the detection of single action potentials across large populations of neurons. Concurrently, advances in microscopy, particularly light-sheet techniques like remote refocusing and oblique plane microscopy, have pushed volumetric imaging rates to over 200 Hz, allowing researchers to capture cellular-resolution neural activity across approximately 25,000-33,000 neurons (25-33% of total brain) in head-fixed zebrafish brain even when at maximum firing rates (Wang et al., 2023).

(Neuropeptides) Optical approaches for monitoring neurotransmitter dynamics are particularly well-suited for larval zebrafish given their transparency and accessibility for whole-brain imaging. Several GENIs have been successfully validated in zebrafish for glutamate (Marvin et al., 2013), GABA (Marvin et al., 2019), acetylcholine (Borden et al., 2020), dopamine (Sun et al., 2018), noradrenaline (Feng et al., 2019), and ATP (Wu et al., 2021), but

developing sensors for the more than 100 neuropeptides identified in the zebrafish brain (Van Camp et al., 2016) remains a challenge.

(Perturbation) The larval zebrafish represents an attractive model organism for optogenetic perturbation due to its transparency and amenability to genetic modifications. One-photon optogenetic approaches face limitations from light scattering, lack of z-axis resolution, and unwanted visual stimulation of the fish (Chai et al., 2024). Two-photon optogenetics offers improved spatial precision and reduced scattering (Turrini et al., 2024), though reliable single-neuron manipulation remains a technical challenge. Current approaches typically target anatomically-defined regions rather than individual neurons, particularly during periods when the fish is relatively stationary (Chai et al., 2024; Turrini et al., 2024). Despite these technical limitations, optogenetics studies in the fish have led to a number of discoveries (Piatkevich and Boyden, 2023), including neural populations and activity patterns responsible for saccadic eye movements (Schoonheim et al., 2010), for increasing sleep (Oikonomou et al., 2019), for controlling swim turn direction (Dunn et al., 2016), for providing sensory feedback to spinal circuits during fast locomotion (Knafo et al., 2017), for producing coordinated swimming patterns (Ljunggren et al., 2014), for stopping ongoing swimming (Kimura et al., 2013), and for contributing to movement in response to noxious stimuli (Wee et al., 2019). Most recently, Chai et al. developed a system capable of enabling whole-brain calcium imaging in freely swimming larvae while simultaneously allowing targeted optogenetic stimulation of specific brain regions during stationary periods (Chai et al., 2024).

Drosophila

(invasive) The Drosophila brain's small size and partial transparency suggest the theoretical feasibility of whole-brain imaging at single-neuron resolution. In practice, however, the presence of air-filled tracheae creates significant challenges through different mechanisms: for single-photon imaging, light scattering is the primary limitation, while for multiphoton approaches, optical aberrations from the tracheae are the dominant obstacle (Hsu et al., 2018). Indeed, two-photon microscopy -- capable of imaging at depths approaching 1 mm in mouse brain tissue (Helmchen & Denk, 2005) -- struggles with imaging depths beyond 0.1 mm in Drosophila due to the air-filled tracheae (2P typically achieves 1mm in mouse cortex) (ref) . While removing air from the tracheae (degassing) could enable whole-brain two-photon

imaging at cellular resolution, this approach is incompatible with normal brain function as it kills the fly (Hsu et al., 2019).

In head-fixed preparations, current techniques navigate this challenge through different trade-offs: light field microscopy achieves fast (~100Hz) imaging of a large portion (600 x 300 x 200 μm3, ~45%) of the brain though at sub-cellular resolution (Aimon et al., 2019). Schaffer et al. used swept confocally-aligned planar excitation (SCAPE) microscopy to image the dorsal third of the Drosophila brain (450 × 340 × 150 μm^3, 29%) at single-cell resolution at 8-12 Hz, though scattering makes resolving individual neurons in deeper brain structures difficult (Schaffer et al., 2023). Two-photon microscopy offers superior penetration power, but its point-scanning nature creates an inherent trade-off between imaging speed and volume coverage. Recent work has pushed these limits by achieving cellular resolution imaging of more than half (~665 x 333 x 245 μm^3, ~68%) of the brain volume at 1.8 Hz (Brezovec et al., 2024). Three-photon microscopy appears to be the most promising approach for achieving true whole-brain imaging with single-cell resolution, as it exhibits reduced scattering and aberration effects, particularly when combined with adaptive optics. However, successful implementation of this approach for comprehensive whole-brain imaging remains an ongoing challenge (Hsu et al., 2019).

In freely-moving or flying Drosophila, neural recording capabilities are more limited. Two-photon calcium imaging systems compatible with tethered preparations have been developed (Seelig et al., 2011) and successfully used to record from small numbers of neurons – such as 4-5 pairs of descending neurons (Schnell et al., 2017) – during wing flapping and steering behaviors that closely mimic natural flight, though these techniques remain restricted to highly constrained experimental conditions.

Another key challenge is achieving sustained recordings over behaviorally-relevant timescales. Several innovative approaches have recently emerged to tackle this challenge. Huang et al. developed a cranial window preparation that allows repeated imaging sessions over up to 50 days by using laser microsurgery to create and then reseal an optical window in the cuticle (Huang et al., 2018), while Aragon et al. demonstrated continuous three-photon imaging through the intact cuticle for up to 12 consecutive hours, though this was limited to bright, superficial neurons (Aragon et al., 2022). Most recently, Flores-Valle et al. achieved long-term

calcium imaging by collecting 30-second recordings every 5 minutes over a 7-day period. This intermittent imaging strategy allowed them to accumulate over 15 total hours of calcium imaging throughout the week-long experiment (Fores-Valle et al., 2022). These long-term imaging approaches are however typically focused on recording from small numbers of neurons, prioritizing stability and duration over coverage.

(Voltage, Patch Clamp, MEA) Progress is also being made in voltage imaging, where several genetically encoded voltage indicators have been applied to Drosophila brain imaging, including ArcLight, ASAP, Ace2N-mNeon, Varnam, and more recently, Voltron (Jin et al., 2012; Yang et al., 2016; Gong et al., 2015; Kannan et al., 2018; Abdelfattah et al., 2019). Using light field microscopy with the ArcLight indicator, Aimon et al. achieved fast (200 Hz) voltage imaging across a considerable portion (no specifics provided in the paper) of the brain at subcellular resolution, though with fewer extractable activity components compared to calcium imaging due to lower signal-to-noise ratios (Aimon et al., 2019). Indeed, voltage imaging faces even greater technical challenges than calcium imaging due to poorer signal-to-noise ratios, and the same limitations regarding imaging depth and resolution apply.

(Neuropeptides) Several GENIs have been successfully validated in Drosophila, including sensors for acetylcholine (Jing et al., 2018; Borden et al., 2020), dopamine (Sun et al., 2018; Sun et al. 2020), and serotonin (Wan et al., 2021). A 2009 review identified 119 predicted neuropeptide precursor genes in Drosophila, with 46 neuropeptides biochemically confirmed from just 19 of these precursors (Clynen et al., 2009); more recent work suggests approximately 50 neuropeptide precursor genes and a similar number of peptide GPCRs (Nässel and Zandawala, 2019). In any case, significant progress remains to be made in developing tools to monitor these neuropeptides' dynamics in vivo.

(Perturbation) The adult fruit fly represents an attractive model organism for perturbation studies, having a well-characterized connectome, relatively small number of neurons (~140,000), and extensive genetic toolkit for targeted manipulation. Two-photon and three-photon optogenetic perturbation benefit from high cuticle transmission in the infrared range, though remain primarily limited by aberration and scattering from gas-filled tracheae rather than the cuticle itself (Hsu et al., 2019). Two-photon stimulation is currently the most commonly used approach, though its effectiveness decreases with imaging depth as scattering

and aberrations increase the effective excitation volume. Somatic targeting is less effective in flies due to their spike initiation sites being distant from the soma (Fişek and Jeanne, 2021). However, the fly brain contains several highly organized regions (like the antennal lobe, mushroom body, and central complex) where dendrites from different neurons occupy distinct spatial territories, providing reasonable spatial selectivity since they are both dense and serve as the main sites of signal integration. While true cellular resolution remains challenging, recent work has demonstrated stimulation resolution of $5 \times 5\ \mu m^2$ laterally and <20 μm axially at the level of antennal lobe glomeruli (Zanon et al., 2022). Most recently, Robie et al. developed the "Fly Disco" system enabling automated high-throughput optogenetic manipulation and behavioral analysis of freely walking flies (Robie et al., 2024). This system combines programmable multi-color LED arrays for optogenetic stimulation with machine learning-based tracking of 21 body landmarks, allowing precise quantification of complex behaviors including locomotion, grooming, and social interactions.

Despite these technical limitations, optogenetics studies have revealed neural populations and activity patterns responsible for a range of Drosophila behaviors (Piatkevich and Boyden, 2023): acquired feeding preferences (Musso et al., 2019), chemotactic navigational decision-making (Hernandez-Nunez et al., 2015), courtship control (Seeholzer et al., 2018), sleep promotion and locomotor activity suppression (Guo et al., 2016), long-lasting internal states in the female brain that regulate multiple behaviors (Deutsch et al., 2020), touch signal processing (Tuthill and Wilson, 2016), context-appropriate walking programs (Bidaye et al., 2020), complex behavioral sequences (Vogelstein et al., 2014), and heading direction representation through ring attractor dynamics (Kim et al., 2017).

Notably, while the recently reconstructed FlyWire connectome provides a complete map of anatomical connections, it does not directly reveal how strongly neurons affect each other's activity in vivo. Pospisil et al. propose that perturbation studies could enable recovery of what they term the "effectome" - a quantitative model of the causal interactions between neurons during brain function (Pospisil et al., 2024). Their key insight is that while studying all possible pairwise interactions between 140,000 neurons would be intractable, the connectome's extreme sparsity (only 0.01% of neuron pairs form synaptic contacts) provides a powerful prior: neurons without direct anatomical connections are unlikely to have strong direct causal

effects on each other. This dramatically reduces the number of potential interactions that need to be measured through perturbation experiments in order to recover the fly's "effectome".

Mice

(invasive) Recent years have seen remarkable progress in our ability to record neural activity in behaving mice, yet fundamental tradeoffs remain between the number of neurons that can be recorded simultaneously, temporal resolution, and freedom of movement. Current approaches to calcium imaging in mice can be broadly divided into head-fixed and freely moving preparations, each with distinct advantages and limitations.

In head-fixed preparations, mice are typically positioned on treadmills that allow some degree of movement while maintaining the stability needed for high-quality imaging. This approach has enabled increasingly comprehensive recordings of neural activity. The MICRONS consortium, for example, recently demonstrated simultaneous recording of approximately 75,000 excitatory neurons across layers 2-5 of visual cortex at 6-10 Hz, spanning multiple visual areas during 14 80-minute sessions over 6 days, close to 20h in total (MICROnS Consortium, 2024). Even more expansive capabilities have been shown using another 2P-imaging approach (light beads microscopy), which has enabled simultaneous imaging of up to 1 million neurons across the dorsal cortex while maintaining cellular resolution (Manley et al., 2024). In parallel, developments in surgical approaches have enhanced optical access - curved glass windows ("crystal skull") that replace the dorsal cranium remain viable for imaging at least 11 weeks post-surgery, providing access to an estimated 800,000-1,100,000 neurons spanning over 30 neocortical areas, though the maximum sustainable recording durations possible with this approach have not been systematically characterized (Kim et al., 2016). However, despite these technological advances, head-fixed preparations fundamentally limit the range of natural behaviors that can be studied.

Advances in freely moving imaging have also been substantial, driven particularly by the development of lightweight microscopes. The MINI2P microscope weighs under 3g and uses a flexible 0.7mm fiber bundle cable, enabling two-photon imaging at 15-40 Hz from 300-600 neurons per plane across up to four imaging planes (approximately 1000 neurons total) within a $420 \times 420\ \mu m^2$ field of view. When stitching across multiple fields of view to cover a $2.2 \times 2.2\ mm^2$ area, over 10,000 neurons can be recorded, though at reduced temporal resolution (Zong

et al., 2022). Even lighter microscopes have since been developed, such as the 0.43g TINIscope (Xue et al., 2023). However, imaging systems compatible with freely moving mice face particularly harsh tradeoffs between the number of neurons recorded, temporal resolution, and signal quality.

(Voltage, Patch Clamp, MEA)
Even with optimal recording conditions, calcium indicators remain too slow to capture individual action potentials for many neurons in mice, particularly for those neurons firing at rates up to 200 Hz. Recent advances in voltage imaging offer promise for tracking individual spikes, with new approaches enabling simultaneous recording from over 300 spiking neurons in the mouse cortex at 400 Hz for over 20 minutes (Bai et al., 2024).

(MEA) A promising example of such an effort ist the international brain laboratory, an initiative that was able to collect a "comprehensive set of recordings from 115 mice in 11 labs performing a decision-making task with sensory, motor, and cognitive components, obtained with 547 Neuropixels probe insertions covering 267 brain areas in the left forebrain and midbrain and the right hindbrain and cerebellum". Mice were trained to turn a wheel in response to the position of a visual grating. This generated roughly 60 Gb of electrophysiology data and 1 Tb of video data, of roughly 150 hours in total.

(Neuropeptides) Several genetically-encoded indicators now enable monitoring of key neuromodulator systems in the mouse brain, including sensors for acetylcholine, dopamine, histamine, norepinephrine, and serotonin (Muir et al., 2024). Additionally, sensors have been developed for select neuropeptides including CRF, dynorphins, enkephalins, GRP, orexins, oxytocin, and somatostatin. However, these tools cover only a small portion of known neuromodulatory systems. Mass spectrometry studies have identified between 500-850 peptides in the mouse brain, with roughly half being classical neuropeptides derived from about 40-45 neuropeptide precursor genes, and half being peptides derived from intracellular proteins (Fricker et al., 2010; Zhang et al., 2012). Developing tools to monitor this broader range of signaling molecules remains an important challenge for understanding neuromodulation in vivo.

(Perturbation) Two-photon optogenetics is the most common method. It can achieve cellular-scale targeting but true single-cell resolution remains challenging - the effective volume of optogenetic-driven spiking is often larger than a single neuron, particularly in the axial dimension – and has been shown to be effective up to layer 5 of cortex. Three-photon excitation offers another approach that can reach nearly 1.5mm into the brain, but requires much higher pulse energies which may limit activation to relatively small populations of neurons. (Lee et al., 2020; Adesnik and Abdeladim, 2021;) Nonetheless, these capabilities have enabled a number of discoveries, including neural populations and activity patterns responsible for a range of diverse behaviors (Piatkevich and Boyden, 2023). These behaviors include parental care (Kohl et al., 2018), spatial object recognition (Kempadoo et al., 2016), aggression against intruders (Lin et al., 2011), breathing rhythm (Sherman et al., 2015), social memory (Oliva et al., 2020) and social-spatial association formation (Murugan et al., 2017), visual perception (Lee et al., 2012), wakefulness (Cho et al., 2017), locomotion (Hagglund et al., 2013), sleep (Kitamura et al., 2017), face gender discrimination (Afraz et al., 2015), water (Zimmerman et al., 2016) and food consumption (Nectow et al., 2017), responses to odors (Root et al., 2014), movement (Gritton et al., 2019) and memory-guided eye movements (Acker et al., 2016), aversion or preference of a place (Kim et al., 2019), reward-seeking (Otis et al., 2017), parental behavior FEF inactivation with improved optogenetic methods | PNAS(Stagkourakis et al., 2020) and encoding of places (Zhang et al., 2013). Most recently, Suthard et al. demonstrated that optogenetic stimulation of hippocampal engram cells can recapitulate the cellular activity patterns seen during natural fear memory recall, revealing coordinated neuron-astrocyte dynamics that underlie both naturally and artificially induced fear states (Suthard et al., 2024).

Humans

(Non-invasive) Current human datasets performed on up to 7T fMRIs contain 16-35h / subject (Tang et al 2022, Allen et al., 2021) at ~1mm^3 resolution, with the most "intensive" datasets reaching 200h /subject (Kupers et al., 2024, Courtois neuromod ref). Norman et al. (2021) achieves a 0.4mm^3 resolution with fUS for 8cm^{3}volume in monkeys. Different groups generated multimodal datasets, such as fMRI and EEG (Mayhew et al., 2013; Pisauro et al., 2017), or fMRI-fNIRS-EEG (Scarapicchia et al., 2017)

[Stand-in] Figure X. Datasets of non-invasive methods already collected, and (highlighted) the datasets we suggest to collect.

(invasive)

(Voltage, Patch Clamp, MEA)

(Neuropeptides)

(Perturbation)

Connectomics & Neuroplasticity: Brain structure reconstruction

As discussed in the previous chapter, single-neuron whole-brain functional data is limited to a few small organisms. Yet, it is possible to reconstruct highly detailed *static* structural images of a brains - and then use the structural data in conjunction with correlated neuro activity data to predict function — structure-to-function.

In order to build an accurate structural representation of the brain, “image resolution should be sufficient to distinguish and trace the finest axon fibers, to identify the nature of

connections [..] and to distinguish them from other forms of adhesion junctions," according to the brain connects workshop series 2021' executive summary notes.

In other words, the minimal structural representation of the brain sufficient to replicating biological information processing digitally is the network of all connections between neurons, i.e. the connectome. The smallest unit here is either synapse - between 200 and 800 nm wide (Sheng and Kim, 2011), the gap between two synapses is about 20-30 nm wide (Yang & Annaert, 2024), or gap junctions between neurons - approximately 2nm. The smallest (unmyelinated) axons are between 50 and 1,000 nm (Helmstaedter et al., 2013) in width and can reach from the motor cortex to the lower parts of the spine.. This necessitates resolution at the single digit to low double digit nm scale. And yet, the human brain contains many billions such interactions spanning over a volume equivalent to about ten tennis balls. For mammalian brains, where most of the neuronal communication is believed to causally travel via synapses rather than gap junctions, the connectomics community typically cites resolution requirements between 10-20nm per voxel to resolve synapses properly (that is each pixel in an image should represent a 3D cube of the brain of the dimensions of say 20x20x20 nm) (Jefferis et al., 2023). Dense connectomics refers to the analysis of all neurons and their connections within an analyzed volume. This is often accompanied by non-neural cells such as astrocytes, microglia, oligodendrocytes, and vascular cells. Staining for these approaches often utilizes chemicals that broadly bind to biomolecules within the samples (such as lipids) to produce recognizable subcellular divisions. Sparse connectomics on the other hand captures a subset of the neurons.

Figure to illustrate the sizes of neurons, synapses and Gap junctions

Iascone, 2020
mouse primary somatosensory cortex
(A) Sparsely labeled layer 2/3 pyramidal neuron (PN). Scale bar: 500 μm. Inset, higher magnification of the neuron shown.
(E) Neuron trace of (A) Scale bar: 50 μm.
(F) volume from inset in (E) with identified inhibitory synapses labeled in white, corresponding trace, overlaid spine annotations, and inhibitory synaptic annotations associated with nodes within the trace. Scale bar: 2 μm.

Find some figure for higher resolution Synapse / gap junction

Santiago Ramón y Cajal's pioneering work established synapses as the cornerstone of neuroscience - a view later confirmed by electron microscopy in 1956 (Jekely and Yuste, 2024). Electron microscopy development reached resolutions less than ten nanometers in the 1930s and, in 1944, broke 2 nm resolution (Haguenau et al., 2003), far surpassing light microscopy constrained to a lateral resolution of ~250 nm and an axial resolution of ~550 nm by the diffraction limit (Huang et al., 2010).

The first connectome, a complete map of all neurons and their synaptic connections to each other, was imaged by South African biologist, Sydney Brenner, in the 1980s. It was Brenner who first thought to do this "radical experiment," according to a 2020 article in *Cell*:

> "...might it be possible to obtain the complete wiring diagram of an animal's nervous system by serially sectioning it into many exceedingly thin slices, imaging each of these sections at high resolution with an electron microscope (EM), and painstakingly tracing each neuron's branches and synaptic connections with other neurons? This audacious idea became reality in 1986 when Brenner, John White, and several other extraordinary scientists produced a 340 page magnum opus, "The Structure of the Nervous System of the Nematode *Caenorhabditis elegans*" (with the running head "The Mind of a Worm")..."

Brenner's work was decades ahead of its time. The worm connectome had to be reconstructed by hand because digital image processing tools at that time were inadequate. Brenner and his colleagues at Cambridge's Laboratory for Molecular Biology imaged and reconstructed all 302 neurons of the highly conserved worm neurons combining 8 different individuals, tracing and connecting each neuron's spindly branches by hand. An intuitive way of illustrating this process is to compare it to satellite images of the earth (see figure below): Not only do you need to take images at extremely high resolution, you also need to determine (encircle individual areas and colorize) and annotate objects (save meta information) in order to make the map useful. The first worm connectome paper has been cited thousands of times and, in almost every year since its publication more than three decades ago, the rate of citations has increased.

Still - it took neuroscientists nearly 40 years of work to go from a worm connectome to a fruit fly connectome. These two organisms have about a three order-of-magnitude difference in the number of neurons.

Santiago Ramón y Cajal himself, despite his groundbreaking work in neuroscience, initially believed that brain circuits were immutable after development. This "static brain" doctrine persisted until the 1960s and 1970s, when researchers began accumulating evidence for continuous structural and functional modifications in the adult brain. Neurons make new connections and abandon unused synapses. Some areas even withstand the trend of declining neuron numbers over life and we see new neurons emerge. Neuronal activity and other variables impose changes on the connectome which influence long-term information processing and thus represent the structural equivalent of learning over time. Modern studies have revealed multiple timescales of structural plasticity, from rapid synaptic modifications occurring within minutes to slower processes of axonal and dendritic remodeling that can take days or weeks. Understanding the rules governing these structural modifications may be as crucial as mapping the connections themselves, as these rules determine how the network can reconfigure itself in response to experience and environmental demands. To lean into our satellite image of the world again: we want to have a dynamic video of the world, rather than a single static picture.

As in the previous section on Neurodynamics, we will start by discussing the prevalent methods in the field of connectomics, followed by data science challenges and then present what has been achieved so far in different organisms.

Current Google Maps maximum zoom is approximately 1 pixel ≈ 15cm on Earth (Google, 2014) (intelligence experts estimate that the most advanced US military satellites can achieve resolutions of perhaps 6-10 cm per pixel. (Richardson, 2024)). So this brain scanning resolution (1 pixel 1.6 cm) would be roughly 10 times more detailed than the best commercial satellite imagery currently available. Or put differently: scanning the human brain at the resolution necessary to reconstruct a connectome is the same as having a map of the world sharper than the resolution in the image below (source).

6 cm
15 cm
60 cm

satellite images
structures, buildings, etc.
addresses, business names, etc.
raw EM image data
neuron segmentation
annotations

Figure X. Brain maps at various stages: following registration (left), following segmentation and tracing (center) and following annotation (right). Source: Schlegel, 2024

Figure: Transitioning from a static, potentially monochrome picture, to a "living picture" in color. The monochrome picture represents morphologic approaches constrained to visualize the contours of cells. Color represents annotated connectomes, e.g. by using expansion microscopy in conjunction with additional antibody labeling. Multiframe videos represent imaging techniques such as 2-Photon imaging that can capture the movement of neurons over time.

Methods of reconstructing the brain

Reconstructing brain structure at resolutions necessary to derive the connectome pushes the boundaries of imaging techniques. On a high level, various imaging approaches use electron beams and various forms of electromagnetic waves- ranging from hard x-rays to visible light- and make use of one or multiple tissue processing techniques such as staining, tissue expansion, or molecular annotations. All methods for static brain structure imaging perfuse the brain with chemicals to stabilize its structure during euthanization, after which it is carefully extracted and respective subsequent tissue preparation steps. The purpose of such tissue processing varies from increasing visibility of important structures to compensating for the resolution limitations of imaging techniques like x-ray or standard light microscopy.

The figure below demonstrates how quickly even rare errors tracing neurons across the extremely large number of images can interfere with the successful reconstruction of a neuron (a 1mm long axon imaged with the above mentioned axial resolution of 20nm comprises 50,000 images). Tissue loss and damage increases the difficulty of following neurons across

different sections during final reconstruction. Even brains of relatively small organisms need to be sliced to arrive at processable sizes that match equipment capacity, diffusion speeds and parallelization across multiple machines. So far, 1mm thick samples have routinely demonstrated robust results. However, cutting brain tissue into ~1mm slices with "ultra-smooth" vibratomy becomes increasingly difficult with bigger brains. For this reason, barcoding techniques emerged in recent years that augment tissues by giving individual neurons unique molecular identifiers to reliably track them across images.

The correlation between neuronal activity and structure unsurprisingly leverages similar methods to the invasive single neuron resolution techniques we discussed in the previous chapter.

Figure: Number of Neurons reconstructed per $1M (inflation adjusted to 2025). This plot uses best estimates on the end-to-end reconstruction costs (sample preparation & slicing, scanning, reconstruction & proofreading) for the four major connectomics initiatives of the past 40y, C. elegans, Fruitfly, Zebrafish and the ongoing BRAIN Connects Mouse connectome

Organism	Year	Time (y)	Neurons	Approximate inflation adjusted Costs($)	Reconstructed Neurons / 1 Million $	Cost / Neuron	Reference
C. elegans (White et al 1986)	1986	10	302	$5,000,000	60	$16,556	Estimate
Fruitfly Zheng et al, 2018 (Murthy, Seung, et al., 2024)	2018	5	140000	$30,000,000	4,667	$214	Estimate
Zebrafish (Svara et al., 2022)	2021	5	100000	$10,000,000	10,000	$100	Conversation with authors
Mouse (NIH, 2024)	2024	5	3000000	$33,000,000	90,909	$11	BRAIN Connects Initiative fundin

Figure Illustration from Brain Connects Workshop Series. It is describing deteriorating accuracy given high numbers of imaging sections with large brain volumes.

Morphology staining approaches

Morphology staining approaches are the most common method in connectomics. Tissue sections are completely stained to highlight morphological features, particularly cell boundaries (membranes) and protein densities. The advantage is that "everything" is visualized and the technique is general to any type of tissue. The disadvantage is that imaging at high enough resolution to resolve desired features is time consuming and data intensive. Classically electron microscopy connectomics uses osmium stains, and light microscopy antibody based morphology staining as well as x-ray synchrotron microscopy has become possible by using high degrees of expansion microscopy.

Electron microscopy (Established)

(How it works) The electron beam, accelerated through a high voltage, is precisely guided by electromagnetic lenses to focus onto the sample with nanometer precision. As the electrons interact with the sample's atoms, they produce various signals, such as secondary electrons, backscattered electrons, or transmitted electrons, depending on the type of microscopy. These signals are detected by specialized sensors, processed, and transformed into highly detailed images, revealing the sample's structure, composition, and even atomic arrangements.

(costs / device and hour of operation, number of globally available devices) Only a handful devices with the capability to scan large brain volumes - as discussed below - exist globally. Setup costs range between $0.5-10M and have similar maintenance costs over 3-5y. Costs per hour accordingly range between $1,000-5,000, depending on uptime.

(Spatial resolution, penetration depth and neuronal level) The Wellcome Trust Scaling connectomics report (Jefferis et al., 2023) provides an excellent discussion of the state of the art processing pipelines in Electron Microscopy. In short, today's most advanced pipelines have a semi-automated process for loading Osmium stained samples into the microscope to scan a surface area of ~150 x 150 μm and then move stepwise across the whole sample. Then, a destructive tissue removal called ion-milling removes a layer off the top of the sample, e.g.

8-16nm. This way a resolution of 16x16x16nm per voxel or lower can be achieved, which represents the gold standard in terms of tissue resolution. Assuming a sample is 1mm thick, after every 100 µm of sample removal by the ion milling process, a replanarization of the sample is necessary. (imagine that the ion-milling process creates a mountainous landscape which gets flooded and replanarized). From there the process repeats. Alternative approaches use hot knife sections which section away layers of the sample - see figure below.

(both sampling rate and upper bound of time sampling) As early as 2015, Zeiss produced 61-beam SEMs capable of approaching GHz data acquisition speeds. (Zeidler et al, 2015). Current state-of-the-art devices have 91-beams. Without going into the details a single beam can be thought of as a focused electron probe that scans a small region of the sample to produce an image of the underlying tissue. The introduction of multi-beam systems dramatically accelerates the imaging process by enabling parallel acquisition of data across a broader area. Each beam operates independently, capturing high-resolution images of its designated section, which are then computationally stitched together to form a comprehensive dataset. Despite burst imaging speeds of up to 3.6 GHz (40Hz / beam), the effective rate projected on a 24/7 scanning time due to downtime, sample exchange etc. falls down to 100-200 MHz. 1Hz in this context is equivalent to imaging a single voxel per second. To put this in perspective: considering that a typical voxel spans x mm3, scanning a mouse brain would take 20 EMs about three years at these speeds, assuming 24/7 scanning.

Replication of Wellcome Trust figure, data see here: The imaging speeds and volumes for electron microscopy in connectomics have increased substantially over time.

(compatability with labelling of different structures beyond neurons, e.g. receptors)

(Data size and storage requirements) At the above mentioned resolution, 1 mm^3 of brain tissue generates approximately 1 petabyte of raw data.

(Data post-processing and quality control)

(frontiers) In 2019, Zeiss produced a 331-beam SEM with only "[e]xchanging a limited number parts" to present as a minimal 2-pager at a conference (ref). This prototype has a 250,000x250,000 nm (hexagonal) field of view with a 5x5 nm resolution, greater than the resolution typically necessary for connectomics. It was made for the semiconductor industry, without any customizations for biology which could potentially further increase performance. Directly extrapolating from best-case 61-beam speeds to the 2019-version with 331 beams gives ~5.4 GHz (1 GHz * 331/61). This prototype is outdated by more than half a decade: Much state-of-the-art technology is proprietary and confidential. It is unclear how far this technology has advanced since, though experts think up to [1000?] beams are not implausible.

From (Jefferis et al., 2023)

Figure 6: Pipeline overview
This figure illustrates the basic steps required for generating a mouse connectome. Importantly, the steps are highly interdependent: e.g. improvements in imaging will likely lead to higher quality alignment and better segmentation.

Expansion Microscopy with Dense Labeling (Maturing)

Brief History: In 2015, Chen et al. introduced expansion microscopy (ExM), a revolutionary technique achieving initial resolutions as fine as 70 nm. Electron microscopy (EM) is constrained by physics to a resolution limit of 0.23 nm (Penczek, 2011), whereas traditional light microscopy is constrained to a lateral resolution of ~250 nm and an axial resolution of ~550 nm by the diffraction limit (Huang et al., 2010). Expansion Microscopy effectively circumvents the diffraction limit by physically expanding the tissue sample itself rather than attempting to improve the microscope's resolving power. Since then, multiple authors demonstrated the dense labeling of protein (M'Saad and Bewersdorf, 2020; M'Saad et al., 2022) and lipid components (Karagiannis et al., 2019).

Process as of today: Most advanced ExM pipelines physically expand biological samples up to 20-fold and beyond by embedding them in swellable hydrogels (see figure). Brain tissue sections (typically 70 μm thick) are first fixed with formaldehyde and acrylamide, then embedded in a first hydrogel containing a cleavable crosslinker (crosslinker help maintain the relative positions of molecules during expansion). After denaturation for 4 hours, the sample expands ~5-fold in water. The sample is then re-embedded in a second neutral gel, followed by a third expansion gel with a non-cleavable crosslinker. After cleaving the first crosslinker, proteins can be labeled with antibodies and pan-stained with fluorescent dyes to reveal ultrastructure. The final expansion in the figure M'Saad et al 2022 achieves ~24-fold total enlargement, enabling imaging of ~15 nm features using standard confocal microscopes. The entire process takes 4-5 days from initial fixation to final imaging, with the key advantage that no specialized equipment - such as expensive electron microscopes - beyond a standard confocal microscope is needed.

Figure by M'Saad et al 2022: pan-ExM-t workflow for mouse brain tissue sections. (a-f) Experimental workflow. (g): Timeline summarizing the protocol. Abbreviations: FA: formaldehyde; AAm: acrylamide; NaOH: sodiumhydroxide; DHEBA: N,N′-(1,2-dihydroxyethylene)bis-acrylamide; SDS: sodium dodecyl sulfate; PBS-T:0.1% (v/v) TX-100 in PBS; ROI: region of interest.

a
Fix, extract, and section mouse brain
a-1
Perfuse whole mouse transcardially with FA/AAm fixative
a-2
Remove brain & fix overnight in FA/AAm
a-3
Make 50-100 µm-thick sections on vibratome
b
Embed brain section in 1st expansion hydrogel
b-1
Incubate in gel monomer solution for 30 min at 4°C
b-2
Secure on flat surface, seal, and polymerize for 2 h at 37°C
c
Denature with SDS and heat
75°C
c-1
Peel hydrogel off the chamber
c-2
Denature in SDS buffer, pH 6.8 at 75°C for 4 h
c-3
Wash in excess PBS
d
Expand ~5x and and re-embed in 2nd and 3rd hydrogels
8 mm
8 mm
15 mm
15 mm
d-1
Expand ~5x in ultrapure water
d-2
Cut ROI with razor blade
d-3
Re-embed in neutral hydrogel
d-4
Re-embed in a new expansion hydrogel
d-5
Incubate in NaOH to cleave DHEBA
e
Pan-stain and expand ~24x
e-1
Incubate in antibody solutions
e-2
Pan-stain with fluorescent dyes
e-3
Wash with PBS-T
30 mm
30 mm
e-4
Expand in ultrapure water to ~24x
f
Image with confocal microscopy
f-1
Acquire images
f-2
Invert color table
g
1 day
Fixation
3 h
1st gelation
4 h
Denaturation
1 h
Wash
2 h
1st expansion
30 min
ROI excision
3 h
1st re-embedding
3 h
2nd re-embedding
1 h
Crosslinker cleavage
3 days
Immuno-labeling
2 h
Pan-staining
2 h
2nd expansion
1 h +
3D imaging

ExM Figure by Eon Systems

SOTA and most recent achievements: Progress has been made towards dense labeling of mm^3 sized tissue in combination with expansion microscopy to achieve EM-like contrasts. Two approaches are non-specific labeling of proteins (pan-protein) using NHS-ester-dyes (M'Saad et al., 2022) (Tavakoli et al 2024[2]) and labeling of lipid membranes (Shin et al., 2024). LICONN showcased proof-of-concept reconstruction of 79 neurons, showcasing ~20 nm lateral and ~50 nm axial spatial resolution (Tavakoli et al 2024[3]). Yet, hydrogels have limitations, distortions and errors.

Tavakoli report their imaging rate at 17MHz, roughly an order of magnitude below current electron microscopes, though lightsheet microscopy is probably two orders of magnitude cheaper than current EMs in use ($80k vs $8M). Given somewhat higher operational costs for processing samples due to antibody staining and more laborious steps, total effective costs for data acquisition could be similar with ExM as compared to EM.

[2] https://www.biorxiv.org/content/10.1101/2024.03.01.582884v2.full
[3] https://www.biorxiv.org/content/10.1101/2024.03.01.582884v2.full

[Stand-in] Figure X. Comparison of raw image data in serial section transmission electron microscopy (top) and expansion confocal light microscopy (bottom). Note the 1-2 order of magnitude higher resolution. Image from [Collins et al., 2024](#).

Upper bounds of existing tech, implications for humans, potential optimizations: Possibly, combining pan-protein staining with lipid-staining and further improvements might improve contrasts in expansion. The major advantage of connectomics based on dense labelling in light microscopy would be that

- expansion microscopy is compatible with quasi arbitrary molecular annotation.
- only a single imaging round is required, compared to the multiple required for barcoding (but see later for concerns about the data analysis when reconstructing neurons).

X-ray synchrotron approaches (Experimental)

X-ray synchrotron approaches use high-energy x-rays to visualize tissue architecture and cellular features. The method can leverage the natural contrast between different cellular

components based on their electron density and elemental composition for minimal preparation requirements, or use heavy metal stains like osmium or gold to enhance contrast of specific features, particularly membranes and synaptic proteins. These stains also increase acquisition time (Ahn et al., 2013, Depannemaecker et al., 2019). Special chemicals can be leveraged to increase radiation resistance of samples (Bosch et al., 2023). Imaging is also generally non-destructive, meaning that the same sample can subsequently be imaged through other modalities.

Spatial resolutions – not limited by diffraction, but rather by the x-ray cross section of elements with low atomic numbers in unstained samples and, more generally, by the risk of damage caused by high x-ray radiation doses – down to roughly 10 nm are theoretically possible in frozen hydrated samples (Howells et al., 2009). The primary advantages are the high penetration depth of x-rays allows for imaging of large tissue volumes (several mm^3) at resolutions approaching 30nm.

The drawbacks are also multiple, however. X-ray approaches for structural brain mapping are far less developed than their EM and LM counterparts, in part because imaging generally requires access to a beamline at a synchrotron facility, with beamlines being generally heavily oversubscribed. Scalability-wise, although simulations are encouraging, significant progress is needed – particularly in terms of improved detectors – before scalability could be sufficient for imaging whole mammalian brains (Collins, 2023, Du et al., 2021). In any case, the field is generally progressing quickly. Recent developments include sub-100 nm imaging and dense reconstruction of fly and mouse brain tissue using x-ray holographic nano-tomography (Kuan et al., 2020), correlative studies involving in-vivo recordings, x-ray synchrotron microtomography and electron microscopy (Bosch et al., 2022), the establishment of the SYNAPSE consortium for imaging a whole human brain at 300 nm resolution (Stampfl et al., 2023), the development of protocols for highly multiplexed x-ray fluorescence (Strotton et al., 2023) and successful imaging of individual synapses through x-ray ptychography (Bosch et al., 2023).

From Dyer et al 2017: Synchrotron X-ray tomography of a millimeter-scale brain sample. A

schematic illustration of the imaging setup is displayed along the bottom: from left to right, the synchrotron X-ray source interacting with an embedded sample of somatosensory cortex as it is rotated during the collection of multi-angle projections. To collect this projection data, X-rays are passed through a scintillator crystal that converts the energy into visible light photons. These photons are then focused onto a light camera sensor, before a sinogram is generated via data collection from a row of sensor pixels. In the three panels above, visualizations of the neocortical sample preparation (a), location of the mounted sample within the instrument (b), and conversion and focusing of X-rays into light photons (c).

Barcoding approaches

As we will see in the following chapter, tracing neurons via morphology relies on the high resolution and subsequent time-consuming reconstruction and error correction. If it were possible to uniquely identify the same neuron — at both its soma and at its far end — that would loosen the hampering constraint of requiring virtually error-free tracing. This is the idea of barcoding: lace a uniquely identifiable molecule in each neuron that can unambiguously determine the identity of each cell. Then, even if it weren't possible to trace a neuron continuously through tissue, the expressed barcode would allow correct identification of distal synapses. In fact, hypothetically loss of an entire section or slicing errors would be much less

catastrophic than in any morphologic approach, as barcoding would not rely on perfect traceability.

Barcodes can come in many forms and scientists are becoming increasingly creative generating more ambitious variants. Also, they can be combined with expansion microscopy to ease readouts. We will focus on two major subtypes, namely using unique codes of nucleic acids or proteins. It is important to note that whether state-of-the-art barcoding methodology are able to capture most neurons and especially most synapses will need to be verified in statistical comparisons against "ground-truth" electron microscope data in small, similar reconstructed samples.

Protein barcoding (Experimental)

History: Protein barcoding for connectomics emerged from the convergence of two key technological advances: site-specific DNA recombination and fluorescent protein engineering. The watershed moment came with Brainbow in 2007, which leveraged Cre/lox recombination to stochastically express different ratios of fluorescent proteins in individual neurons. This approach achieved roughly 100 distinguishable color combinations through the differential expression of red, green, and blue fluorescent proteins. The initial proof-of-concept in mice demonstrated the potential for unique cellular labeling, though it also revealed fundamental challenges in achieving consistent expression levels and maintaining color fidelity across large tissue volumes. Expression in different cell types is uneven, trafficking of these proteins is uneven, and fluorescent staining is uneven. Within the same neuron, a distal axon may display a slightly different barcode than the soma, complicating correct identification. Subsequent iterations expanded the technique to other model organisms, notably Drosophila, while attempting to address these limitations through improved fluorescent proteins and more sophisticated genetic designs. (ref, ref, ref, ref)

Process as of today: The most advanced approaches combine three key elements: genetic targeting, protein-based labeling, and multi-round imaging. In current protocols, viral vectors deliver genetic constructs encoding multiple protein markers, each under the control of independent promoters. These markers can be fluorescent proteins, epitope tags, or engineered

protein scaffolds designed for subsequent antibody labeling. (see figure by Bramlett et al). The overall process requires the multiple rounds of staining, washing, and imaging required.

Such barcoding approaches face three distribution problems when delivery via viral vectors:

1. Distribution to all neurons.
2. Even distribution among all neurons.
3. Distribution within neurons.

Figure barcoding [Bramlett et al 2020]

Status quo with most recent achievements: In 2020 Shen et all combined Brainbow with multi-round immunolabeling in expansion microscopy allowed the unique identification of neurons and reconstruction of their structure, including imaging up to 15 different stained targets. (ref) Notably, super-multicolor Tetbow bei Leiwe et al 2024 allows reconstruction despite two sections of a neuron being separated, beginning to leverage the core strength of

barcoding. (ref) In principle, methods like Tetbow could scale to whole-brain connectomics when combined with expansion microscopy, pan-protein and/or lipid-staining.

As of now achieving whole brain connectomics using barcoding is not possible though. The research focused research organization (FRO) E11 is actively working on advancing barcoding technologies towards whole mammalian brains.

Upper bounds of existing tech, implications for humans, potential optimizations: The theoretical limits of protein barcoding intersect with fundamental biological constraints and technical capabilities. In mammalian brains, where individual neurons form between 8,000 and 30,000 synapses, the mathematics of unique identification become particularly challenging. With a 25-bit binary code (2^25 combinations), statistical analysis reveals that in a human brain of 80 billion neurons, approximately 10 million neurons (0.02%) would share both a barcode and at least one synaptic connection. While this error rate might seem problematic, it compares favorably with current electron microscopy reconstruction error rates, particularly when considering that spatial information can help resolve ambiguous cases.

[First estimate]

$$C_{\text{total}} = \frac{N_{\text{neurons}}}{N_{\text{barcodes}}} \times S_{\text{neuron}} \times N_{\text{neurons}} = \frac{8 \times 10^{10}}{2^{25}} \times 30,000 \times 8 \times 10^{10} \approx 72.8 \times 10^{6} \text{ (collisions)}$$

[Second estimate, to be doubled checked]

1. Probability of synapse sharing

$$P(\text{shared}) = \frac{C_{\text{total}}}{N_{\text{pairs}}} = \frac{8 \times 10^{10} \times 6,000}{\frac{(8 \times 10^{10})^2}{2}} \approx 1.5 \times 10^{-7}.$$

2. Conditional probability of sharing a barcode given synapse sharing

$$P(\text{barcode} \mid \text{shared}) = \frac{P(\text{barcode}) \times P(\text{shared})}{P(\text{shared})} = \frac{1}{2^{25}} \approx 3.03 \times 10^{-8}.$$

3. Total number of collisions

$$C_{\text{neurons}} = C_{\text{total}} \times P(\text{barcode} \mid \text{shared}) = (4.8 \times 10^{14}) \times (3.03 \times 10^{-8}) \approx 1.45 \times 10^{7}.$$

Reaching almost all neurons with the viral vectors carrying the barcodes is difficult. This will require further breakthroughs in delivery, such as the intravenous bCap1 AAV capable of

reaching 5-20% of cells in the brain. (ref) Various virus-based platforms (lyssavirus, Sindbis, HSV, etc.), better intravenous delivery, high-density intra-CNS injections, and creative approaches to multi-site injection throughout the brain will be required — or perhaps all four simultaneously. Combining approaches make 90% coverage seem hard but feasible with extensive technology development.

It is noteworthy that nucleic acid barcoding approaches exist, where unique DNA barcodes (typically 15-30 nucleotides) are introduced into neurons using viral vectors or genetic engineering and and then uses sequencing as a readout. The field emerged from spatial transcriptomics methods developed in the 2010s. The transition to connectomics applications began with MAPseq (Kebschull et al. 2016), which introduced high-throughput projection mapping - tracing where neurons from one brain region send their axons, without detailing individual synaptic connections. For connectivity attempts (BRICseq): Although not yet working at scale, there are current efforts to barcode neurons and transform connectomics from an imaging problem to a sequencing and analysis problem (Huang, 2020). Most recently, ExBarSeq (Alon et al. 2023) combined barcoding with expansion microscopy to improve spatial resolution to ~20nm. However, these methods remain most powerful for projection mapping rather than detailed connectivity analysis. Current throughput for projection mapping reaches 100,000+ neurons per experiment, while detailed connectivity mapping remains limited to hundreds of neurons due to challenges in reliably getting barcodes to and reading them from synapses.

Structural time series data methods (Neuroplasticity)

For such approaches a cranial window - essentially a small piece of transparent skull replacement that provides access into the brain - is necessary. These windows can last from months to over a year with proper maintenance (ref). However, the window's optical clarity tends to degrade over time due to bone regrowth, tissue healing responses, and protein deposits.

Two-photon (endo)microscopy

Calcium Imaging

Super-resolution microscopy

Data Science

Storage & Bandwidth

While high-throughput imaging of mammalian brains demands extraordinary effective bandwidths, existing technology – especially when combined with modern compression methods – can largely meet these requirements.

A human brain is roughly 1000-1400 cm^3, and such a volume, if imaged at 10 nm isotropic resolution, represents a total of roughly 1.2 zettavoxels. Assuming 2 bytes per voxel and a total acquisition time of about a year, grayscale imaging thus requires a total effective bandwidth of about 600 terabits/s. Multiplexed imaging scales bandwidth requirements linearly with the number of colors; thus, streaming of 30-color imaging data requires a total bandwidth of roughly 18 petabits/s. However, state of the art compression methods already demonstrate a 128x reduction in data size without compromising subsequent reconstruction (Li et al., 2024). Thus, data compression at the source could see effective bandwidth requirements decrease to about 140 terabits/s for 30-color imaging and to about 5 terabits/s for grayscale imaging. Furthermore, the connectome can be eventually summarized through connectivity graphs and neuron skeletons instead of a 3D voxel representation, which would still preserve the most critical information and lead to roughly a million-fold reduction in storage size [source].

[Stand-in] Figure X. Effective total bandwidth requirements for human brain imaging in less than one year at 10 nm isotropic resolution. (over one year)

Bandwidth requirements thus represent an important challenge, particularly if no compression is used. However, they are not far beyond what existing technology already enables in fields such as high energy physics. Indeed, experiments within the LHC at CERN can produce roughly a petabyte (that is, 8 petabits) of raw data per second, with various filtering and compression algorithms within the sensor decreasing the effective data rate to a more manageable 50 terabytes (400 terabits) per second (Radovic et al., 2018). Such high data rates of course require highly performant networking infrastructure, but that too is not far beyond current capabilities: Google data centers, for example, have demonstrated within-datacenter bandwidths of up to 13 petabits/s (Vahdat, 2024). As long as compression is used and processing happens for the most part within a data center co-located with the imaging facility, imaging bandwidth should thus not represent a fundamental bottleneck to efforts aiming to image a whole human brain in roughly a year, provided the level of investment is sufficient.

Depending on the compression factor the data storage necessary for a human connectome would be on the order 1 Peta to 100 Exabytes, with additional 2-3x factor to accommodate for backup infrastructure. Petabyte storage capacity is common for small to mid-sized datacenter. Most large datacenters operated by commercial providers don't disclose their official capacity, but can store up to several exabytes (10^18) of data (Blackblaze 2024). The European Centre for Medium-Range Weather Forecasts (ECMWF) generates 400 terabytes of new data daily and processes this in conjunction with previous dates to make predictions (ECMWF, 2024). Companies like ByteDance process over 500 PB/day (Wu et al., 2024) while the biggest actors such as Google, Meta or Microsoft don't disclose official numbers. For 2025, the total globally available digital storage capacity is estimated to be in around 16 zettabytes (16E21) (Statista Inc., 2021).

Figure compute and storage trends
Both figures are replicated.
Figure for Storage costs of a graph representation of a connectome

Data storage: Current storage prices fell to ~10$/TB and lossless image compression algorithms can reduce the image size by 15x without impairing tracing quality. (Minnen et al., 2021).

Data processing:

Neuron Reconstruction

After all the image data has been collected for every voxel of the brain, neuron reconstruction begins. The goal of reconstruction is to recreate the 3D connectome from microscopy data. Reconstruction consists of three main steps: data preparation, tracing, and proofreading.

As mentioned in the introduction of this chapter Brenner et al were forced to capture individual images and manually trace individual neurons over the course of several weeks. Because, despite a suite of AI tools known as "flood-filling" algorithms can trace thousands of neurons in parallel, that single component — the quality controlled tracing of neurons — did not scale sufficiently fast, it took years to reconstruct the fruitfly's neurons despite substantial computational support (and a total of 40 person years).

Registration (aligning adjacent scans) is the critical bottleneck during data preparation. Misalignments are caused by artifacts in EM scans and slight movement/rotation of samples across imaging rounds. Robust registration ensures spatial consistency of structures, and poor alignment during the data preparation step is the dominant cause of errors in automated neuron reconstructions [Popovych et al., 2024]. Recent advancements in machine learning have significantly improved alignment accuracy by addressing (non-exhaustively) artifact removal, de-warping, and noise-reduction, in some cases reducing genuine misalignments to 0.06% [Popovych et al., 2024] [Scheffer et al., 2020].

After data preparation is segmentation of cell boundaries and tracing of neuron skeletons. This step is how neuron shapes and connectivities are reconstructed. Manual tracing and segmentation are prohibitively costly averaging 11.2 hours per neuron [Zheng et al., 2018]. The primary consequence of poor segmentation is the cost of human labor for proofreading, which can be upwards of $1bn (Jefferis et al., 2023). Automated segmentation techniques like U-Nets can learn from sparse training data and perform well on general tasks like segmentation and synapse classification. Flood-filling networks (FFNs) are the state of the art in segmentation and offer superior segmentation performance (>99.5% precision, which measures "of all positive predictions, how many were correct?") [Januszewski et al., 2018]. Though FFNs have high computational costs, architecture modifications can reduce

computation time by orders of magnitudes with comparable performance [Januszewski et al., 2018].

Besides model architecture, there are considerable gains from generating more ground truth data through proofreading. Currently, only about 1% (601 neurons) of the 1 mm^3 MICrONs dataset has been proofread (ref). Similarly just 100/~50,000 neurons in the H01 human temporal lobe dataset have been proofread (Jefferis et al., 2023). It wouldn't be difficult to incentivize large-scale proofreading for these datasets. Proofreading efforts have been augmented using a combination of machine-suggested edits, region-definition, and morphology libraries for comparison, which together have sped up proofreading times by several orders of magnitude (100-1000x) [Scheffer et al., 2020, Plaza, 2016].

Given enough data AI has approached, achieved or surpassed human-level performance on many conceivable benchmarks. This holds true across most of humanity's points of pride in cognitive achievement: Chess (Campbell et al., 2002), Go (Silver et al., 2016), reasoning (OpenAI, 2024), parts of mathematics (Trinh et al., 2024), software development (Schluntz et al., 2024), natural language (Brown et al., 2020), reading comprehension (Rae et al., 2021), and visual reasoning (OpenAI, 2024).

Figure Tracing speed

Image reconstruction and Computational models: ~10,000x Speed increase of neuron reconstruction from image data over time over time. [Flesh this out. Give specifics from timepoint A vs. timepoint B.] Assumption: 1mm^3 mouse brain tissue, 27,972 Sections, 200,000 Cells, 5400 m of neurite length ➕ Automatic Tracing Costs

Figure Superhuman visual AI
Figure: AI becomes superhuman in many tasks, including vision, particularly specialized visual tasks. By Our World in Data

Datasets

[elaborate on existing structural datasets, similar to the analysis for functional data above]

Over the last four decades, scientists have reconstructed the entire connectome for 13 organisms. Note that this is not 11 different *species*, but literally 11 individual animals. Ten connectomes are from the worm, *C. elegans*, and the eleventh, twelfth and thirteenth are from the sea squirt *Ciona intestinalis* (Ryan et al., 2016), from the larva of the marine annelid *Platyneresis dumerilii* (Verasztó et al., 2024) and from the fruit fly respectively. The full connectome of one zebrafish has also been scanned, but those images haven't yet been reconstructed.

By Organism

Figure scale of brains

Caption: Since the first connectome was reconstructed in 1985 for C. elegans, substantially larger areas of brain tissue have now been acquired and computationally emulated. The figure provides both a sense of scale as well as a list of most sophisticated emulations for the respective brain tissues.

C. elegans

(Structural mapping) The simplicity and stereotypy of C. elegans neuroanatomy made it the first organism to have its complete connectome mapped. In 1986, White and colleagues published the landmark reconstruction of the hermaphrodite nervous system, compiling electron microscopy data from multiple specimens (at least four adults and one L4 larva) to map about 5000 chemical synapses, 2000 neuromuscolar junctions and 600 gap junctions (White et al 1986). This achievement was possible because C. elegans neurons maintain remarkably consistent positions and connectivity patterns across individuals (Varshney et al., 2011).

This initial mapping was later complemented by the reconstruction of the male-specific nervous system, which contains approximately 80 additional neurons primarily involved in

mating behaviors (Jarrell et al 2011). A comprehensive re-mapping effort by Cook et al. (2019) produced updated connectomes for both sexes, correcting earlier errors and introducing synaptic strength weights based on measuring how many consecutive tissue sections each synapse spanned in the electron microscope images (Cook et al 2019). More recent work has expanded into developmental neurobiology, with reconstructions across five different developmental stages (Witvliet et al 2021), while continuing refinements driven by recent advances in neuron identification, alignment, processing, and scale have improved both accuracy and completeness (Skuhersky et al 2022, Brittin et al 2021). To date, C. elegans remains the organism with the most individual connectomes reconstructed – approximately ten complete C. elegans brains. However, due to the highly stereotyped nature of C. elegans neural connectivity, the marginal scientific value of additional individuals may be limited compared to other organisms with more variable nervous systems.

While electron microscopy provided the foundational C. elegans connectome maps, these structural maps alone do not capture the rich molecular complexity that supports the worm's behaviors. Despite having only 302 neurons, C. elegans relies heavily on sophisticated molecular machinery at each synapse - with the worm's postsynaptic proteins amounting to about half the number for mammals (Emes et al., 2008). To understand this molecular complexity, optical approaches like expansion microscopy have emerged as crucial complementary tools. The first C. elegans-specific expansion microscopy protocol (ExCEL) enabled both immunostaining for molecular identification and tissue expansion through innovative cuticle permeabilization techniques (Yu et al., 2020). With standard ExCEL achieving 3.5x expansion and iterative ExCEL reaching up to 20x expansion (25nm resolution), these methods may resolve individual synaptic connections while preserving the critical molecular information that EM cannot capture.

(Storage and processing requirements, neuron reconstruction) Given a total body volume of 2-6 million μm^3 for an adult hermaphrodite worm, imaging at 10nm isotropic resolution generates a total of 2-6 x 10^{12} voxels. At 2 bytes per voxel for a single channel, this would require 4-12 terabytes of storage, scaling linearly with the number of colors. Historically, White et al. attempted to use a Modular I computer in the 1970s for computer-assisted reconstruction, but ultimately resorted to manual annotation of printed electron micrographs using colored Rapidograph pens to trace neurons through serial sections. The tracing and reconstruction of

the C. elegans required years of painstaking work, with the project as a whole taking 15 years to complete. More recent efforts such as Cook et al. have leveraged specialized software tools like Elegance (Xu et al., 2013), resulting in speedups of several orders of magnitude, and recent advances in machine learning and computational processing have sped up the process further.

(Structural time series efforts) [What efforts/methods should we make sure to discuss here?]

Drosophila

(Structural mapping efforts) Synaptic resolution electron microscopy efforts in Drosophila achieved an early milestone in 2015 with the complete imaging of a female first instar larva nervous system (Ohyama et al. 2015), followed by another major advance in 2018 with the complete imaging of an adult female fly brain using a custom high-throughput serial-section transmission electron microscopy (ssTEM) platform (Zheng et al, 2018). This was followed by focused-ion-beam scanning electron microscopy (FIB-SEM) imaging of the female hemibrain (Scheffer et al 2020) and, most recently, electron microscopy imaging has extended beyond the central brain with the mapping of the female (Azevedo et al., 2024) and male (Takemura et al., 2024) ventral nerve cords.

Beyond electron microscopy, expansion microscopy (ExM) has emerged as a complementary approach for Drosophila connectomics. When coupled with lattice light-sheet microscopy, ExM has enabled the comparison of neural circuit connectivity across multiple specimens while preserving molecular contrast information (Gao et al., 2019). This protein-specific molecular labeling capability has been further enhanced through transgenic approaches like Bitbow, which enables combinatorial protein barcoding to uniquely label neurons. By targeting five spectrally distinct fluorescent proteins to three subcellular compartments (membrane, nucleus, and Golgi apparatus), Bitbow has demonstrated the ability to generate up to 32,767 distinct molecular barcodes for studying neural circuits in the fly brain (Li et al., 2021). Most recently, advances in expansion microscopy using potassium acrylate-based hydrogels have achieved expansion ratios exceeding 40x, enabling light microscopy visualization of features like mitochondria within presynaptic compartments at resolutions approaching those of electron microscopy while maintaining whole-brain coverage (Tian et al., 2024). X-ray based techniques also offer another avenue for large-volume imaging, with X-ray holographic

nano-tomography (XNH) achieving 140-170 nm resolution across millimeter-scale volumes of Drosophila nervous tissue (Kuan et al., 2020).

(Storage and processing requirements, neuron reconstruction) Assuming a brain volume of 0.08 mm^3, imaging at 10nm isotropic resolution generates approximately 8 x 10^13 voxels. At 2 bytes per voxel for a single channel, this requires approximately 160 terabytes of storage per channel.

The progression of Drosophila neuron reconstruction reflects both advancing capabilities in automatic neuron tracing and machine-assisted reconstruction proofreading. The initial complete brain imaging by Zheng et al. demonstrated feasibility with a proof-of-concept reconstruction of 120 neurons, with reconstruction requiring, on average, 11.2 person-hours per neuron (Zheng et al., 2018). This was followed by a significant improvement in the hemibrain project (Scheffer et al., 2020), which reconstructed over 22,000 neurons and 20 million synapses using automated segmentation supplemented by human proofreading. The recent FlyWire achievement (Murthy, Seung, et al., 2024) represents another leap forward, with reconstruction of the complete central brain containing 140,000 neurons and more than 50 million synapses. Parallel efforts have expanded beyond the central brain, with reconstruction of approximately 15,000 neurons and 45 million synapses in the ventral nerve cord (Azevedo et al., 2024).

(Neuroplasticity efforts)

Larval Zebrafish

(Structural mapping efforts) Early electron microscopy efforts in larval zebrafish demonstrated the feasibility of whole-brain imaging through multi-scale imaging approaches. Hildebrand et al. achieved imaging of an entire larval zebrafish brain and portions of the spinal cord, though at resolutions insufficient for dense reconstruction (Hildebrand et al., 2017). A major advance came with Svara et al., who achieved whole-brain electron microscopy imaging at synaptic resolution in a 5-day post-fertilization larval zebrafish, enabling tracing of neural connections across nearly the entire brain except for the retinae (Svara et al., 2022). Parallel efforts are ongoing, including as part of integrated functional & structural studies that imaged another whole larval brain (Anonymous authors, 2024) and as part of work at the Brain Science and

Intelligent Technology Innovation Center of the Chinese Academy of Sciences, where a team led by Du Jiulin is combining both optical and electron microscopy approaches for cellular and synaptic level mapping respectively (Du Jiulin, 2022). Small pieces of the brainstem (Vishwanathan et al., 2024) and spinal cord have also been reconstructed (Svara et al., 2018).

Beyond electron microscopy, expansion microscopy (ExM) has emerged as a powerful complementary approach, first demonstrated in zebrafish by Freifeld et al. who resolved putative synaptic connections between fluorescently labeled cell populations (Freifeld et al., 2017). Subsequent work has revealed detailed protein organization at specific synapses, such as those on Mauthner cells (Cárdenas-García et al., 2024). Recent ExM protocols enable the expansion of intact, several-millimeter-long animals up to 5 dpf while maintaining compatibility with genetically-encoded protein labeling, providing an essential link between structure and function by revealing both subsynaptic structures and intricate signaling pathways throughout the nervous system (Steib et al., 2023, Behzadi et al., 2024). While X-ray based approaches have been demonstrated using synchrotron radiation to achieve cellular resolution (Osterwalder et al. 2021), they have not yet been widely adopted for neural circuit mapping in larval zebrafish. Barcoding has not been demonstrated in the Zebrafish yet.

(Storage, processing and neuron reconstruction) The Hildebrand et al. dataset requires approximately 2.7 terabytes of storage across all resolution levels, while the Svara et al. dataset, likely requires approximately 15 terabytes of storage (assuming 2 bytes per voxel at 7.33 teravoxels, the size of the current effort is ~11TB at 8bit) (mapZebrain, 2025). Neuron reconstruction efforts for the larval zebrafish are ongoing: while automated segmentation and synaptic detection of the Svara et al. dataset have been completed, comprehensive proofreading efforts continue to this day and are scheduled to be completed at the end of 2025 (Personal communication, mapZebrain, 2025). This positions larval zebrafish as a frontier organism from structural mapping efforts: the only published organism where whole-brain electron microscopy imaging at synaptic resolution has been achieved but proofreading work remains to be completed (unpublished work in other insects exists as per conversation with several authors).

(Neuroplasticity mapping efforts)

Mouse

(Structural mapping efforts) The establishment of the IARPA MICrONS consortium in 2016 marked an important development in mouse connectomics. The work of the consortium progressed in two major phases - first imaging a focused volume in cortical layer 2/3 (250 x 140 x 90 μm), then expanding to image and reconstruct an entire cubic millimeter spanning six cortical layers and three higher visual areas. MICRoNS combined in-vivo calcium imaging with electron microscopy, generating over a petabyte of imaging data (MICrONS Consortium et al., 2021). During this period, a parallel effort by Motta et al. mapped a 90 x 90 x 60 μm volume of somatosensory cortex within just 4,000 person-hours (Motta et al., 2019).

In 2023, the NIH launched the $150 million BRAIN Initiative Connectivity Across Scales (BRAIN CONNECTS) program, funding 11 projects over 5 years to develop tools for brain-wide connectivity mapping. A key project within this initiative, led by Harvard University in collaboration with Google Research, Allen Institute, MIT, Cambridge University, Princeton University, Johns Hopkins University, received $33 million to reconstruct and proofread 10 mm^3 of the mouse brain - specifically the hippocampal formation, a region crucial for memory and spatial navigation. While focusing on just a fraction (roughly 2-3%) of the mouse brain, this volume represents a notable scaling challenge, being approximately 10 times larger than the previous MICrONS dataset. The five-year project will use two 91-beam electron microscopes operating in parallel. Success would demonstrate whether current electron microscopy and automated reconstruction technologies can scale sufficiently to eventually tackle the complete mouse connectome. Notably, maintaining a similar 5-year timeline for imaging an entire mouse brain would require scaling to approximately 40-50 such microscopes working in parallel - highlighting both the technical challenges and infrastructure requirements for mapping complete mammalian brains at synaptic resolution.

When it comes to expansion microscopy in the mouse brain, the availability of sophisticated genetic tools combined with the prohibitive scale of EM-based reconstruction has accelerated development of alternative approaches. E11 Bio's recently announced PRISM platform integrates genetic barcoding with expansion microscopy by expressing unique combinations of protein tags in each neuron - currently achieving hundreds of thousands of distinct molecular

signatures with plans to scale to billions - which are then visualized through multiplexed immunostaining in expanded tissue (E11 Bio, 2024). Among other ExM developments, ExA-SPIM (Glaser et al., 2024) demonstrated unprecedented imaging throughput, achieving 946 megavoxels per second with an effective resolution of 250x250x750nm after 4x expansion - though this resolution is not yet sufficient for dense connectomic reconstruction. The team successfully imaged entire 3x expanded mouse brains in just 24 hours per channel, tracking sparsely labeled subcortical projection neurons and their axonal projections across the brain. This combination of high throughput, multi-color capability, and relatively low system cost (~$250,000) makes it particularly promising for whole-brain mapping efforts. This potential has motivated the development of new optical systems, including a custom lens that, when combined with higher expansion factors of 3-12×, will enable effective lateral resolutions of 50-150nm - potentially bringing the system into the resolution regime required for dense reconstruction. Meanwhile, Tavakoli et al with their LICONN (light-microscopy based connectomics) approach demonstrated dense reconstruction in mouse cortex, imaging a ~1 million cubic micrometer volume spanning cortical layers II/III-IV at effective resolutions of ~20nm laterally and ~50nm axially through ~16-fold expansion, with the 0.47 teravoxel dataset acquired in just 6.5 hours at an effective voxel rate of 17 MHz (Tavakoli et al., 2024).

X-ray microscopy efforts are also multiple. Early demonstrations using X-ray holographic nano-tomography (XNH) achieved sub-100nm resolution across 300x200x1000 μm tissue volumes (Kuan et al., 2020). More recently, Bosch et al. developed a correlative workflow combining in vivo calcium imaging, synchrotron X-ray tomography, and volume electron microscopy to investigate both function and structure within the same tissue (Bosch et al., 2022). In follow-up work, Bosch et al. also demonstrated another key advance - using X-ray ptychographic tomography under cryogenic conditions with specialized radiation-resistant resins to achieve sub-40nm resolution capable of resolving individual synapses (Bosch et al., 2023). While whole-brain X-ray imaging has been demonstrated at cellular resolution (Humbel et al., 2024), achieving synaptic resolution across large volumes remains an active area of development.

(Storage, processing and neuron reconstruction) The mouse hippocampus connectome is expected to generate an estimated 25 petabytes of data (Google Research, 2023). Imaging a whole mouse brain (approximately 500 mm^3) at 10nm isotropic resolution would generate

approximately 5 x 10^{17} voxels (or 3.2 x 10^{16} voxels at 25 nm isotropic resolution). At 2 bytes per voxel for a single channel, this would require approximately 1 exabyte (or 64 petabytes). The significant storage requirements of connectomes at the scale of the whole mouse brain (and beyond) have motivated the development of compression techniques capable of alleviating data management constraints: EM-compressor, for example, can decrease the storage needed to store raw EM data by as much as 128x without compromising subsequent neuron reconstruction (Li et al., 2024). Meanwhile, neuron reconstruction efforts are currently ongoing for the MICrONS cubic millimeter containing an estimated 120,000 neurons. Automatic synapse detection has identified over 523 million synapses within the volume and, as of September 2024 (v1181), over 1,400 axons have been manually proofread and cleaned, establishing over 500,000 verified connections to somas within the dataset (MICrONS Consortium, 2024).

(Neuroplasticity mapping efforts)

- https://www.nature.com/articles/s41592-023-01936-6?

Humans

(Structural mapping efforts) Despite the considerable technical challenges of imaging such large volumes of nervous tissue at synaptic resolution, electron microscopy efforts in primate and human brains have nonetheless achieved several notable milestones. Wildenberg et al. imaged a 0.8 mm × 2.4 mm × 40 nm block of rhesus macaque cortex, revealing that primate neurons receive 2-5 times fewer synaptic inputs than their mouse counterparts (Wildenberg et al., 2020). Loomba et al. conducted comparative connectomic analysis across species by imaging multiple cortical samples - including ~175 × 220 × 100 μm volumes of layer 2/3 from macaque and human, and a larger 1.7 × 2.1 × 0.03 mm volume spanning all cortical layers in human temporal cortex (Loomba et al., 2022). A landmark achievement came in 2024 when Shapson-Coe et al. demonstrated high-throughput serial section electron microscopy of a 170-μm-thick slab of human temporal cortex at nanoscale resolution. This volume, obtained during epilepsy surgery, spans approximately 1.05 mm^3 of tissue (accounting for sectioning-induced compression) and was imaged at a resolution sufficient to resolve individual synapses and subcellular structures. Thus, it represents the largest volume of human brain

tissue imaged at a resolution sufficient for dense connectomics to date (Shapson-Coe et al., 2024).

The most significant X-ray imaging effort for human connectomics is the SYNAPSE (Synchrotron for Neuroscience—an Asia-Pacific Strategic Enterprise) collaboration, which aims to map an entire human brain at 0.3 μm resolution (Stampfl et al. 2023) in 2-3 mm thick sections (Chen et al., 2021). While the COVID-19 pandemic significantly disrupted the initiative's launch in 2020, the collaboration has made remarkable technical progress, achieving imaging speeds of 1 mm^3 per minute (a 10-fold improvement over initial capabilities) through developments in unmonochromatized X-ray imaging and automated sample handling that enables week-long continuous acquisition. The project now coordinates roughly 10 synchrotron facilities across Asia-Pacific, including the Brain Imaging Beamline at the Taiwan Photon Source - the first synchrotron beamline entirely dedicated to connectomics (Chen et al., 2021). Their next phase, SYNAPSE 2.0, aims to achieve another 10-100x speed increase to 0.2 mm^3/second, though this will require significant expansion of both the collaboration and computing infrastructure (Stampfl et al., 2025).

(Storage, processing, and neuron reconstruction) The reconstruction of primate connectomes at synaptic resolution remains a massive undertaking. Storage requirements alone would be significant: assuming two bytes per voxel, a typical marmoset brain would require 11.6 exabytes at 10 nm isotropic resolution (0.74 exabytes at 25 nm); a typical macaque brain 208 exabytes (13.3 exabytes); and a human brain roughly 2.3 zettabytes (147 exabytes). The volume imaged by Shapson-Coe et al. – representing an extremely minute part of a whole human brain, or approximately 0.00007% of a whole human brain – produced over 1.4 petabytes of data (Shapson-Coe et al., 2024), motivating the development of dedicated infrastructure (Maitin-Shepard and Leavitt, 2022). Given the scale of this challenge, some whole-brain mapping efforts opt for lower resolution imaging approaches. Even so, storage requirements remain substantial - the SYNAPSE collaboration, which aims to map the human brain at 0.3 μm resolution using X-ray imaging, estimates they will need exabyte-level storage for a single brain dataset and approximately 100 exabytes for their complete connectome mapping goals. Reconstruction would also represent a daunting challenge. The H01 dataset

released by Shapson-Coe et al., even with thousands of Google-developed Tensor Processing Units (TPUs) for automated neuron segmentation and synapse detection methods (Blakely and Januszewski, 2021), still required extensive manual proofreading. While the computational pipeline successfully identified around 57,000 cells and 150 million synapses, ensuring accuracy still demanded intensive human effort, with expert reviewers spending over 3.5 hours per neuron to correct remaining errors. Proofreading efforts continue to this day, with the original release only including 104 proofread cells.

(Neuroplasticity mapping efforts)

Miscellaneous Organisms

Interlude: Multimodal datasets

- Combination of Neurodynamics
- Combinations of structure and structure (Xray + EM + ExM)
- Functional recording then structure, e.g. MICRONs, Zebrafish, others

Computational Neuroscience: Simulating Brains

In the 1940s, inspired by Edgar Adrian's groundbreaking work on the electrical impulses of neurons (see Chapter Neurodynamics), the idea of engineering a physical replica of neural computations might have seemed audacious. At the time, vacuum tubes and analog circuits represented the pinnacle of technology, and the complexity of the brain appeared insurmountable. Yet, in 1951, Marvin Minsky and Dean Edmonds rose to the challenge by constructing the Stochastic Neural Analog Reinforcement Calculator (SNARC). This pioneering machine, powered by vacuum tubes and motorized potentiometers, was the first hardware-based system to embody a multi-neuron network capable of learning through reinforcement. Though SNARC never achieved widespread fame and was never formally published, it demonstrated a profound truth: even a modest array of neuron-like units could adapt its connections to solve tasks, laying the foundation for the field of artificial neural networks.

Since then, countless researchers have followed in Minsky and Edmonds' footsteps, striving to replicate or simulate neural systems in increasingly sophisticated ways. These simulations vary widely in their scope, from the type of simulation (e.g., spiking vs. rate-based models) to the number of neurons and synapses, the hardware and software used, and even the computational slowdown required to simulate one biological second. Despite these differences, the overarching goal remains the same: to capture the essence of neural computation and bridge the gap between biological brains and artificial systems.

However, simulating neurons and synapses is only part of the challenge. For brain models to demonstrate meaningful behavior, they must interact with their environment. This requires embodiment—the ability to encode external stimuli into neural activity and decode that activity into actions. Embodiment doesn't necessarily demand a physical robotic body; it can occur in a virtual environment, where sensory inputs and motor outputs are simulated alongside the brain model. This process mirrors modern brain-computer interfaces or cochlear implants, where information is encoded and decoded into neural inputs and outputs for specific tasks (e.g., seeing, hearing, memorizing, or speaking). Over time, this interaction can improve iteratively, benefiting from adaptive changes in either the emulated brain or the interface. Ultimately, the goal is for the embodied brain emulation to perform tasks that serve as validation criteria, such as...

To explore these ideas further, we will begin by discussing the computational architectures used to simulate neuronal networks and the tools that enable these digital neurons to interact with digital bodies. From there, we will pivot to examine the most relevant and infamous attempts at brain simulation, focusing on specific organisms in subsequent chapters.

Methods of Simulating the BrainComputational Approaches to Neuron and Synapse Modeling

Computational models of neurons and synapses have become indispensable tools for bridging the gap between partial functional measurements, structural wiring data, and the richly detailed behavior of living brains. As discussed in prior chapters, exhaustive single-neuron resolution recordings are often unattainable in large organisms, and connectomes alone are insufficient to explain circuit function. Neuron models, therefore, aim to capture how each cell transforms incoming activity into an output—whether that output is a firing rate, a spike train,

or a graded voltage response. By fitting a model's parameters to observed neuronal behavior, researchers gain both explanatory power and a means to predict how real cells might respond under new conditions.

Neuron Models

Neuron models aim to capture how electrical or synaptic inputs map onto changes in a neuron's membrane potential or spiking. Some frameworks treat neurons as abstract point units with minimal internal dynamics, whereas others explicitly encode multiple ion channels, compartments, or morphological details. A variety of software frameworks exist to facilitate simulations (NEURON; Nego; NeuCube; NeuroGrid; GeNN)

Models	biophysically meaningful	tonic spiking	phasic spiking	tonic bursting	phasic bursting	mixed mode	spike frequency adaptation	class 1 excitable	class 2 excitable	spike latency	subthreshold oscillations	resonator	integrator	rebound spike	rebound burst	threshold variability	bistability	DAP	accommodation	inhibition-indiced spiking	inhibition-induced bursting	chaos	# of FLOPS
integrate-and-fire	-	+	-	-	-	-	-	+	-	-	-	-	+	-	-	-	-	-	-	-	-	-	5
integrate-and-fire with adapt.	-	+	-	-	-	-	+	+	-	-	-	-	+	-	-	-	-	+	-	-	-	-	10
integrate-and-fire-or-burst	-	+	+		+	-	+	+	-	-	-	-	+	+	+	-	+	+	-	-	-		13
resonate-and-fire	-	+	+	-	-	-	-	+	+	-	+	+	+	+	-	-	+	+	+	-	-	+	10
quadratic integrate-and-fire	-	+	-	-	-	-	-	+	-	+	-	-	+	-	-	+	+	-	-	-	-	-	7
Izhikevich (2003)	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	13
FitzHugh-Nagumo	-	+	+	-		-	-	+	-	+	+	+	-	+	-	+	+	-	+	+	-	-	72
Hindmarsh-Rose	-	+	+	+			+	+	+	+	+	+	+	+	+	+	+	+	+	+		+	120
Morris-Lecar	+	+	+	-		-	-	+	+	+	+	+	+	+		+	+	-	+	+	-	-	600
Wilson	-	+	+	+			+	+	+	+	+	+	+	+	+	+		+	+				180
Hodgkin-Huxley	+	+	+	+			+	+	+	+	+	+	+	+	+	+	+	+	+	+		+	1200

A comparison of the neuro-computational properties of neuron models. "# of FLOPS" is an approximate number of floating point operations (addition, multiplication, etc.) needed to simulate the model during a 1 ms time step. Note that in practice, different models can support time steps of varying length while still maintaining a target level of accuracy (Skocik and Long, 2014). Source: Izhikevich, 2004

(Leaky integrate-and-fire) One of the simplest spiking models is the leaky integrate-and-fire (LIF) approach. It treats the neuron membrane as a resistor-capacitor circuit, whose potential u integrates an incoming current while spontaneously decaying (the "leak"), and triggers a spike upon reaching a threshold. The membrane then resets to a resting voltage. This scheme introduces only one differential equation (plus a rule for thresholding and reset), and captures fundamental aspects of spiking behavior. Its computational lightness makes it a staple for large-scale network simulations, although it omits ion-channel kinetics or dendritic geometry (Skocik and Long, 2014).

(Izhikevich) The Izhikevich model is a more flexible single-compartment system with two coupled state variables, typically v for membrane voltage and u for a recovery process. Although it lacks direct biophysical details about sodium and potassium currents, it can reproduce many spike-timing patterns—regular, bursting, chattering—through suitable tuning of just four parameters (a,b,c,d). In practice, Izhikevich neurons are common in spiking network simulations that benefit from more realistic spiking patterns than LIF can provide, but do not require the fully biophysical detail of Hodgkin–Huxley (Izhikevich, 2004; Skocik and Long, 2014). A caveat is that the model's properties can shift as the time step changes, so care is needed to ensure consistent solutions at different step sizes (Skocik and Long, 2014).

(Hodgkin–Huxley) In contrast to these more phenomenological approaches, the Hodgkin–Huxley model preserves explicit ion-channel kinetics derived originally from voltage-clamp experiments on the squid giant axon. Its membrane potential u is governed by separate sodium, potassium, and leak conductances, each described by gating variables (m,n,h) that follow voltage- and time-dependent transition rates. This scheme, although more parameter-intensive, remains an essential tool for replicating the shapes, thresholds, and frequency responses of neuronal action potentials. It can also be adapted to incorporate multiple channel subtypes or altered gating parameters (Skocik and Long, 2014). Beyond single-compartment usage, the Hodgkin–Huxley formalism commonly appears in multi-compartment neuron models, where each compartment has its own channel dynamics.

(Multi-compartment Hodgkin–Huxley) Expanding Hodgkin–Huxley to a multi-compartment framework allows modeling of dendritic branches, axonal initial segments, and other spatial structure. These models capture local dendritic spikes, back-propagation, or region-specific ion-channel gradients, which can be essential for explaining how single cells

integrate inputs. While multi-compartment models certainly can involve dozens or even thousands of compartments (Herz et al. 2006), expert consensus appears to be that many applications need only a small handful—for instance, two to five—to capture soma-dendrite interactions or back-propagating action potentials (Carlsmith 2020). This extra complexity enables phenomena like dendritic coincidence detection, local dendritic plateau potentials, or intricate backpropagation, which can shape the cell's coding properties in ways that single-compartment models cannot capture. Thus, large compartmental expansions represent a rather flexible choice: increasing compartments and parameters can boost realism in dendritic computations, at the cost of heavier per-neuron memory usage and computational flops.

Note that some authors disagree on which models are truly most efficient once accuracy is enforced, with papers by Izhikevich, Skocik and Long, and Valadez-Godinez et al. presenting varied benchmarks and optimization criteria (Izhikevich, 2004; Skocik and Long, 2014, Valadez-Godinez et al., 2019). There is no universal consensus, reflecting how different time steps, solver methods, and accuracy demands can drastically alter measured performance.

Synapse Models

Synapses transmit signals between neurons, exhibit short-term dynamics based on recent activity, and undergo long-term changes during learning. Computational models of synapses vary from simple rules to detailed biophysical simulations, with different models emphasizing different aspects of synaptic function.

(Models of Synaptic Transmission) The simplest models treat each synapse as a fixed weight: presynaptic spikes cause instantaneous jumps in postsynaptic current. While computationally efficient, this ignores that real synapses produce responses that rise and decay gradually over time. More realistic models generate a transient response for each spike, using either an alpha function or difference of exponentials to capture this time course (Roth and van Rossum, 2009). These waveforms can be applied either to conductance (capturing voltage-dependence but requiring more computation) or directly to current. Although these temporal response functions (functions that describe how conductance or current evolve over time) better reflect the time course of synaptic events, they still simplify receptor properties and ignore potential voltage dependence. NMDA receptor-mediated effects can be modeled by including voltage-dependent block of the conductance by magnesium. Going beyond these relatively simple kinetic schemes, more detailed models use kinetic schemes tracking multiple receptor

states (closed, open, desensitized), while the most complex simulations also model neurotransmitter diffusion and geometric effects in the synaptic cleft. However, such detailed diffusion models are typically too computationally demanding for network simulations and are more commonly used to study single synapses (Destexhe et al., 1998).

(Short-Term Plasticity Models) The models described above capture instantaneous synaptic responses, but real synapses show activity-dependent changes over milliseconds to seconds, exhibiting either depression (decreased effect) or facilitation (increased effect) with repeated activation (Citri and Malenka, 2007). Two main phenomenological models capture these effects: the Tsodyks-Markram model treats synapses as having a pool of resources that deplete with use and recover over time, while Abbott et al's model directly modifies release probability (Tsodyks et al., 1998; Abbott et al., 1997). Both models can fit experimental data well despite their simplicity. More mechanistic variants, however, can track multiple vesicle pools, presynaptic calcium, and other factors that drive more complex short-term dynamics.

(Long-Term Plasticity Models) Whereas short-term changes fade within seconds, synapses also undergo longer-lasting modifications that can persist for hours or days. Such long-term plasticity is thought to underlie learning and memory, and is typically modeled through Hebbian-like rules. Basic Hebbian models strengthen synapses when pre- and postsynaptic neurons are active together, but require stabilizing modifications to prevent runaway growth. Spike-timing-dependent plasticity (STDP) implements this principle at the level of individual spikes: synapses strengthen when presynaptic spikes precede postsynaptic ones by tens of milliseconds and weaken for the reverse order. Still, straightforward STDP rules cannot capture frequency-dependent or burst-dependent effects. More advanced models address these nuances through multi-spike interactions (Pfister and Gerstner, 2006) or by simulating how calcium influx drives synaptic changes (Shouval et al., 2002).

Model fitting and Data-Driven Approaches

Regardless of a model's complexity, its predictive usefulness depends on how effectively its parameters are constrained by experimental measurements (Almog and Korngreen, 2016). Historically, most neuron models were constrained by manually tuning a few parameters (e.g. leak conductance, threshold, or channel densities) to match qualitative observations, such as a neuron's typical firing frequency or spike waveform. Modelers would "hand-fit" the neuronal behavior by trial-and-error until the simulated voltage traces or firing rates closely resembled a

reference dataset. Although workable for simple scenarios, this approach often fails to generalize and can obscure parameter degeneracies—different parameter sets may produce similar outputs without revealing which ones are biologically correct.

A second phase arrived with expanded neural recordings—particularly optical techniques that allow simultaneous measurements from large sets of neurons—and with new simulation frameworks that support automatic differentiation. When a model's equations are fully or partially differentiable (as Hodgkin–Huxley usually is), gradient descent can be applied to systematically minimize the mismatch between recorded and simulated activity. This has motivated recent work on differentiable simulators that can backpropagate errors from final spike outputs or time-varying voltage traces through an entire model, yielding parameter sets consistent with optical or electrophysiological data. In other cases where discontinuities or non-smoothness arise (e.g. certain spiking resets), generative methods can learn to map recordings to model parameters without needing direct backpropagation through each spike event.

For synaptic models, patch-clamp electrophysiology remains the gold standard for parameter fitting. Single-cell recordings characterize basic synaptic transmission parameters like postsynaptic current kinetics and receptor properties, while paired recordings or optogenetic stimulation reveal presynaptic release probability and short-term plasticity dynamics. Studying long-term plasticity poses additional challenges, as these changes unfold over hours to days and involve complex molecular cascades that are difficult to monitor in living tissue while maintaining sufficient temporal and spatial resolution.

While such approaches benefit from biologically informed model architectures (e.g., known ion-channel structure or a leaky-integrate-and-fire scheme), one can also relax these biophysical priors entirely and rely on purely data-driven methods. Large recurrent or feedforward neural networks—trained end-to-end on high-dimensional time-series data—can in principle capture dynamics that conventional equations might omit. However, this freedom demands enormous datasets. In smaller organisms or well-defined circuits—like C. elegans or larval zebrafish, where single-neuron resolution data can be collected in large quantities—purely "black-box" models with minimal biophysical assumptions can thrive.

Structure to function

For larger mammalian brains, though, comprehensive, whole-brain functional data remain beyond present technology, making it impractical to learn all parameters directly from raw observations. Consequently, biophysical priors—such as those embedded in multi-compartment Hodgkin–Huxley or simpler spiking models—remain crucial to reduce parameter space and keep simulations biologically plausible. Yet even these more traditional approaches struggle to scale up to billions of cells; far more extensive measurement capabilities would be required to fit every neuron and synapse.

A promising strategy for bridging the structure-to-function gap is to develop generative models that link morphological and molecular data to neuronal function. In smaller, more experimentally tractable organisms such as C. elegans or larval zebrafish, one can gather both a molecularly annotated connectome and rich functional data (e.g., through optogenetic perturbations and whole-brain recordings). By correlating neuronal responses with ultrastructural features—such as synapse size, receptor distributions, and dendritic geometry—these models learn how specific structural attributes translate into core biophysical parameters like channel conductances or short-term plasticity kinetics. Once validated in systems where ground-truth data are abundant, the same approach can be extended to large mammalian brains, where whole-brain functional measurements remain infeasible. In this context, Holler et al stands as a landmark demonstration of how morphological information alone can inform functional predictions: by combining slice electrophysiology with electron microscopy, they showed that synapse size reliably predicts synaptic strength in mouse somatosensory cortex (Holler et al., 2021). Their work exemplifies how structural features can serve as powerful proxies for synaptic function. Incorporating molecular-level data into such pipelines would enable generative frameworks to infer parameters with even greater precision and scale, providing a viable path toward biologically grounded simulations of large neural circuits. The necessary condition for that is a large database of slice synaptic electrophysiology data, such as Campagnola et al 2022.In this way, the integration of high-resolution structural data with targeted functional measurements can progressively narrow—and ultimately bridge—the gap between brain structure and brain function. This will require substantial investments in such aligned datasets.

Embodiment

Accurate neuron and synapse simulations alone cannot ensure meaningful behavior. For a computationally replicated or emulated brain to demonstrate capabilities akin to its biological counterpart, it must be embodied—that is, embedded in a body and environment that allow it to perceive sensory signals (vision, sound, touch, smell and taste) and produce motor outputs of increasing complexity. In practice, this involves two core tasks: encoding external stimuli into neuronal activity (e.g., turning photons into retinal spike trains) and decoding the brain's outputs into bodily actions (e.g., muscle contractions). In highly sophisticated embodiments this would also include hormonal encoding and decoding. Though "body" need not imply a physical robot—virtual organisms in simulated environments can suffice—it must provide consistent two-way interactions that mirror those found in nature.

The different biomechanical body simulations will be discussed in the respective sections for the organisms below. To feed the simulations, either recordings of stimuli from the real world (ref) or virtual environments can be used.

One common engine is Multi-Joint dynamics with Contact (MuJoCo), developed in 2012 and currently owned & maintained by Google Deepmind. MuJuCo is a physics simulator optimized for biomechanics, robotics, and other scientific or engineering uses. Besides scientific engines, video gaming surpassed a market cap of beyond $455B (ref). Driven by the market forces of video games, simulated environments and worlds have been approaching photorealism over recent decades. The very same market forces drove the scale of development of modern-day GPUs enabling single GPUs to render highly detailed graphics at real-time speeds.

(Encoding) One area where encoding external signals into neuron-like activity is of special interest is neuromorphic computing: here, classical Von Neumann computational architectures, consisting of a separate memory and processing unit between which information is constantly moved, are replaced with biologically-inspired units that act as processing and memory simultaneously and communicate via spikes, as biological neurons do (ref). Such a computational architecture requires input information to be encoded into spikes, and recent years have seen large amounts of progress being made, most prominently in the fields of vision (Gallego et al. 2020) and hearing (Donati & Valle, 2024). While the optimal way to encode

information into spikes cannot be answered uniformly (Auge et al., 2021), the efficacy of these methods in encoding signals for embodied systems can be verified experimentally via neuroprostheses (Donati & Valle, 2024) and neurorobotics (Bartolozzi, Indiveri & Donati, 2022).

In animals, linking sensory input with neuronal responses has a long history: the discovery of robust responses of specific neurons to specific stimuli, e.g. vision (Hubel & Wiesel, 1962), location (O'Keefe & Dostrovky, 1971) or head direction (Taube 1998), was one of the driving factors of our current understanding of the brain. More recently, machine learning models of the visual cortex have allowed us to predict the response of large numbers of neurons to dynamic visual stimuli, i.e. movies (Turisheva et al., 2024). Building off of large scale datasets of visual, behavioural, functional, and connectomic data, we are moving closer towards foundational models of vision, models that capture abstract aspects of neuronal processing, can accurately predict neuronal activity to previously unknown stimuli in different areas of the brain, and can be applied to new animals with limited training (Wang et al., 2023). Whether such models accurately encode visual stimuli can also be tested experimentally by extracting stimuli predicted to cause strong activations, and then presenting these stimuli in vivo (Walker et al., 2019).
A similar approach has been used in humans, where activation strength in response to natural language sentences, as measured by fMRI, can be predicted using LLM-based encodings, and strongly activating sentences can be extracted from the model (Tuckute et al., 2024). Recently, there has been some success in uniting data from different recording modalities in humans (EEG, MEG, and fMRI) to create a foundational model capable of encoding and decoding visual stimuli to/from brain activity (Ferrante et al., 2024).

(Decoding) Recent advances in fMRI and EEG decoding demonstrate that language and visual perception can be decoded. For example, when subjects are shown specific images, reasonable facsimiles of these input images can be reconstructed from both fMRI Scotti et al., 2023 (see figure) and MEG Benchetrit et al., 2023 data. Chen et al., 2023 expanded on reconstructing multiple frames, i.e. video from fMRI data. Likewise, efforts to decode the semantic meaning of written or oral text demonstrate that semantic meaning can be extracted from fMRI and

MEG data.

Figure 2. Reconstructed images from fMRI data. 7T fMRI data was collected from subjects examining natural images (Allen et al., 2022); these fMRI data can be "decoded" into images that strongly resemble the input image (image data from Scotti et al., 2024 repository under MIT license).

From [Chen et al., 2023](#))

[Tang, 2022](#) were able to decode speech from fMRI datasets using participants who were listening to short stories. An example from the paper is shown in the following figure.

Figure from [Tang, 2022](#)

Invasive recordings like [Antoniades et al., (2023)](#) [showcase](#) how activity of individual neurons can be mapped to the visual input of a mouse. Also lots of work predicting primate behaviour from neural manifolds extracted from activity, e.g. https://arxiv.org/abs/2202.06159 https://arxiv.org/abs/2205.08413 https://www.nature.com/articles/s41551-021-00811-z

something about EEG - https://www.nature.com/articles/s41598-021-81805-4

Not looked into it closely

Figure: From Neuroformer Paper

Verification

The history of technological advancement is often marked by the establishment of benchmarks. In artificial intelligence, the introduction of ImageNet (Deng et al., 2009) and its associated ILSVRC competition revolutionized the field by providing a standardized dataset and objective metrics for comparing models. While these benchmarks did not settle debates about the nature of "intelligence," they enabled measurable progress by focusing on a tangible proxy: classification accuracy. Similarly, the pursuit of emulating biological brains requires rigorous methods to evaluate success. Without agreed-upon benchmarks, the field risks stagnation, with progress hindered by subjective or incompatible criteria. Standardized evaluations—even imperfect ones—are crucial for aligning efforts, tracking advancements, and accelerating discovery, much as ImageNet catalyzed the rise of deep learning. The central

challenge, therefore, is to design benchmarks that capture measurable aspects of neural or functional fidelity, driving progress until their limitations necessitate the next leap forward.

A foundational framework for evaluation was proposed in Sandberg and Bostrom's Whole Brain Emulation Roadmap (Sandberg and Bostrom, 2008). They distinguished between simulations, which replicate outputs, and emulations, which replicate internal dynamics, arguing that valid models must preserve the brain's causal structure—the step-by-step relationships between neural states. However, practical challenges abound. Even setting aside the brain's potential chaotic dynamics, minor discrepancies—stemming from noise in neural recordings (Rupprecht, 2021) or parameter inaccuracies—can compound over time, causing simulations to diverge from biological trajectories. To address this, Sandberg and Bostrom suggested tolerating deviations smaller than the brain's inherent "noise floor," the variability observed across repeated biological trials. This pragmatic approach underpins one of the field's most advanced benchmarks, ZAPBench (Lueckmann et al., 2024), which evaluates larval zebrafish emulations by predicting 30 seconds of neural activity from 10 seconds of observed calcium traces (sampled at 1 Hz). Models are scored on per-neuron mean absolute error (MAE) across over 70,000 neurons.

An alternative framework shifts the focus from neural activity to behavioral indistinguishability. Zador et al. propose an "embodied Turing test": if a virtual animal navigates novel environments, responds to threats, or learns tasks indistinguishably from its biological counterpart, the emulation succeeds—regardless of internal mismatches (Zador et al., 2023). This mirrors Turing's original vision, replacing conversation with sensorimotor behavior. For instance, an artificial beaver might be tested on dam-building, or a simulated fruit fly on evasive maneuvers during flight. However, this framework remains comparatively underdeveloped, existing more as a conceptual proposal than a fully fleshed-out benchmark.

The lack of consensus in brain emulation mirrors debates in AI. Just as no single metric—ImageNet accuracy, chess Elo ratings, or language modeling capability—fully captures "intelligence," no single benchmark will resolve what defines a successful brain emulation. A promising approach then is to develop benchmark suites that aggregate diverse tasks. Initiatives like BIG-Bench (Srivastava et al., 2022) and HELM (Liang et al., 2022) evaluate language models across hundreds of scenarios, recognizing that no individual task tells the whole story.

For whole brain emulation, a similar suite might combine neural activity prediction (as in ZAPBench), embodied behavior tests, causal perturbation experiments (e.g., optogenetic interventions), and new innovative metrics that the community will develop in the future. Indeed, while benchmark suites and in general the portfolio approach to evaluation discussed here provide a more comprehensive evaluation framework, significant work remains to refine and expand the evaluation landscape. Nonetheless, by iteratively improving and expanding benchmarks the field can advance objectively, grounded in empirical progress rather than abstract debates.

As part of this report simulation attempts discussed for different organisms were rated on the following 0-3 point scale across 10 dimensions. The dimensions gesture at the breadth of sophisticated benchmarks that eventually need to be developed.

	Connectivity accuracy	Percentage of neurons	Cell types	Plasticity	Neuronal Accuracy	Neuromodulation	Temporal resolution	Behavior	Personality-defining Characteristics	Consider Learning horizons?
0	Artificial (no real connectivity data, using random or artificial connections)	Small circuit – substantially less than a brain area.	No cell types (only one neuron type, e.g., like in typical ANNs).	Completely static network structure and synaptic parameters.	-	None (no neuromodulation signaling systems modeled)	No concept of time (ANNs) or very coarse representation of time (like "before" and "after").	None (no emergent behaviors modeled)	None (no personality traits represented)	No Learning
1	Simple statistical rules (inspired by a few biological observations)	A whole brain area or multiple interacting brain areas.	Most basic cell types, e.g., excitatory vs. inhibitory neurons.	Some limited plasticity, such as short-term plasticity.	Neuronal networks (ANNs with units like ReLU, firing rate units, no spiking)	Single modulator (single neuromodulation signaling system like dopamine only)	Slow relative to neuronal spiking activity (hundreds of milliseconds to seconds and more).	Simple outputs (basic stimulus-response patterns)	Basic traits (emerging from simple rules)	Short term
2	Complex statistical rules (from systematic biological studies and/or partial connectomic information)	~Whole brain	Some cell type diversity, e.g., at the neuronal subclass level (such as Pvalb, Sst, Vip inhibitory subclasses or differences between cortical layers).	More extensive plasticity (e.g., STDP).	Point Neurons (LIF)	Multiple modulators (interacting neuromodulation signaling systems)	Consistent with the scale of neuronal spiking activity (time steps in the range of a fraction of millisecond).	Feedback loops (simple feedback between different neural systems)	Scattered traits (emerging from network interactions)	Medium Term
3	Full connectome (complete including verified synaptic connections)	~Whole body	Diverse cell types, possibly including non-neuronal cells.	Full dynamic plasticity including growth and pruning of connections.	Neuronal Compartments	Complex system (interacting neuromodulation systems with feedback)	Fast (microseconds or faster, capturing details of fast subcellular processes).	Complex behaviors (emerging from system interactions)	Complete profile (personality profile emerging from neural dynamics)	Long-term

Computational requirements

(Model fitting computational demands)

(running the model - computational demands)
Get an lower and upper bound for each organism across the computational demands.

MacBook Pro 2024 M4 Pro $4,700
MacStudio M2 Ultra $5,600
NVIDIA A100 SXM $20,000
NVIDIA H100 NVL$25,000
NVIDA DGX GH200 $30,000,000 (estimate)
NVIDIA HGX B200 $350,000

(hardware)

Total compute in supercomputers has been rising exponentially for decades and supercomputers in the 2020s have been reaching exaflop territory, e.g. with El Capitan at more than 1.5 exaflops (Thomas, 2024), and typical costs of $600M (Joseph, 2023). It's worth noting that clusters used for machine learning applications typically do not appear in TOP500 and that, as a result, the computational capacity of state of the art systems likely exceeds these values. For example, in 2023 Google demonstrated the ability to train language models on a cluster of 50,944 TPUv5e chips (for a peak performance of over 10 FP16 exaFLOPs) (Ananthamaran, 2023), and although no public benchmarks have been reported so far, the 100K H100 Colossus cluster recently assembled by xAI would at least theoretically be capable of over 100 FP16 exaFLOPs, with further plans to roughly double the size of the cluster in the coming months (Mantel, 2024).

Compute capabilities: Famously described by Moore's law, compute costs fall at an exponential speed. Data storage costs follow a less steep, but still exponential curve. Since almost all steps of brain emulation from data collection to eventual emulation are computationally-demanding, Moore's law also pays dividends for connectome research.

[Stand-in] Figure X. Memory and interconnect have not kept pace with processing power. Y-axis is normalized peak hardware FLOPS w.r.t. the R10000 processor.

Pricetags as of Jan 2025. Max compute FLOPS / s (FP16 (Half Precision)) Max Storage Bytes Max Interconnect (bits / s)

The widening gap between processing power and memory/interconnect capabilities has created bottlenecks in large-scale neural simulations that will likely require more thorough integration of memory and processing.

Over the past 30 years, the rate of improvement in processing power has far exceeded the rate of improvement in memory and interconnect development. Peak hardware FLOPS have

improved by roughly 3x every 2 years, compared to only 1.6x and 1.4x for memory and interconnect bandwidth respectively, leading to the so-called memory wall (Gholami et al., 2024, An et al., 2024). Memory capacity has also undergone a similar trend: today's El Capitan boasts 1.74 exaFLOPS and over 5.4 petabytes of HBM3 memory (Thomas, 2024); NEC's Earth Simulator boasted 41 teraFLOPS and 10 terabytes of DRAM memory in 2004 (Sato, 2004); representing an approximately 42,400-fold increase in processing power and just a 540-fold increase in memory capacity. Like many other workloads, computational simulations of brain tissue have been affected by this trend: a 2014 study identified interconnect bandwidth as a bottleneck for spiking neural network simulations (Kunkel et al., 2014) and a recent in-depth analysis found memory bandwidth and interconnect latency to represent key bottlenecks for all types of neuron simulations studied – from point neurons with current-based synapses to multicompartmental models with conductance-based synapses – at high fan-ins and neuron counts (Cremonesi et al., 2020).

Although many interventions – such as moving to lower precision arithmetic (Igarashi, 2024) – could help decrease memory and interconnect requirements, most of them would also lead to corresponding increases in processing power – the H100 GPU, for example, sees a boost from 67 teraFLOPS to almost a petaFLOP by switching from double to half precision (NVIDIA Corporation, 2023). Indeed, the effective hardware utilization rate would remain bottlenecked by memory and interconnect bandwidth, much like how data movement and latency bottlenecks are expected to limit the training of frontier AI systems (Erdil and Schneider-Joseph, 2024). Overcoming memory and interconnect bottlenecks thus effectively requires adopting hardware architectures not characterized by the von Neumann bottleneck, such as neuromorphic ones.

Biological neural networks are inherently parallel, asynchronous and event-driven systems. Their computational architecture is fundamentally distinct from conventional von Neumann machines, particularly in how information and storage are implemented. At their core, synapses serve as both computational elements and memory units – a characteristic that cannot be efficiently replicated in systems based on the von Neumann architecture. The physical separation of memory and processing in such system necessitates continuous data movement between those components, resulting in significant energy expenditure – a single off-chip memory access costs roughly four orders of magnitude more than half-precision arithmetic

operations (Jouppi et al., 2021) – and potential performance bottlenecks. The shift toward GPU architectures has partially addressed these limitations through massive parallelization - while CPUs typically contain fewer than 16 cores optimized for sequential processing, modern GPUs like NVIDIA's H100 incorporate tens of thousands of cores for parallel computation (NVIDIA, 2023). This architectural advantage has enabled significant performance gains in neural network simulations, with platforms like NEURON demonstrating up to 100x speedups when migrating from CPUs to GPUs (Zhang et al., 2023). However, biophysically detailed neuron simulations of all types still exhibit substantial performance degradation as the number of neurons and synapses increases, primarily due to bottlenecks in memory and interconnect bandwidth.

These limitations have motivated the development of neuromorphic systems – systems whose architectural design more closely mirrors the organizational principles that characterize biological neural networks. By co-locating memory and processing elements, these systems inherently avoid the von Neumann bottleneck while maintaining massive parallelism comparable to modern GPUs. Crucially, neuromorphic systems also implement asynchronous, event-driven computation, significantly reducing both latency requirements and energy consumption in large-scale neural simulations. The field has seen steady progress, from IBM's TrueNorth in 2014 (implementing 1 million neurons and 256 million synapses per chip) (Akopyan et al., 2015) to Intel's Loihi platforms (Davies et al., 2018, Orchard et al., 2021) and more recent developments like the Hala Point system at Sandia National Laboratories, with over 1.15 billion neurons and 128 billion synapses (Intel, 2024). However, the massive investment in AI accelerators – orders of magnitude larger than in neuromorphic computing – has led to a research landscape where repurposing AI hardware for biological neural network simulations is often the preferred approach due to its widespread availability, mature software ecosystems and sheer raw computational capabilities (Wang et al., 2023, Landsmeer et al., 2024, Deistler et al., 2024). Nevertheless, the fundamental constraints that govern information processing in biological systems are increasingly shaping AI accelerator development, driving convergence toward neuromorphic principles such as co-location of memory and processing (Wolters et al., 2024).

Organism

Better
Worse
C. elegans
Drosophila
Zebrafish
Mouse
Human
Sarma (2018)
Kim et al. (2019)
Simeon et al. (2024)
Zhao (2024)
Huang (2019)
Higuchi (2022)
Cowley (2024)
Lappalainen et al. (2024)
Shiu et al. (2024)
Anonymous (2024)
Liu (2024)
Vishwanathan (2024)
Gertler et al. (2008)
Billeh (2020)
Rodarie (2022)
Schepper et al. (2022)
Wang (2023)
Gambosi et al. (2024)
Jiang (2024)
Moll and Miikkulainen (1997)
Dumoulin & Wandell (2008)
Lytton (2008)
Kasabov (2014)
Verhulst et al. (2018)
Yamazaki et al. (2021)
Lu (2024)
Pronold (2024)
Connectivity accuracy
Percentage of neurons
Neurontypes
Plasticity
Functional Accuracy
Neuromodulation
Temporal resolution
Behavior
Personality-defining Characteristics
Learning

C. elegans

Where is my C. elegans emulation?

(What the argument is, and what the 1986 provided) A common refrain in discussions about whole brain emulation (WBE) is the seeming stagnation of progress despite the early mapping of the C. elegans connectome. Critics often point to the 1986 publication by White et al., "The Structure of the Nervous System of the Nematode Caenorhabditis elegans," (often referred to as "The Mind of a Worm") as evidence that brain emulation, especially of more complex organisms, is simply infeasible. "We've had the C. elegans connectome since 1986," the argument goes, "and still haven't achieved a functional emulation. How can we possibly hope to emulate a human brain?" This perspective, while understandable at first glance, fundamentally misrepresents both the nature of the 1986 connectome and the significant challenges that have historically hindered progress in C. elegans emulation, challenges that are only now beginning to be addressed with modern neurotechnologies. To appreciate the current state of the field, it's crucial to understand precisely what White et al. delivered, and what they did not. Their landmark paper provided a wiring diagram—a map of synaptic connections between neurons, using chemical synapses and electrical gap junctions—and represented the culmination of over a decade of painstaking manual tracing of serial electron micrographs. This connectivity matrix, painstakingly derived from thousands of electron micrographs from multiple individual worms (although neural wiring is largely conserved across individuals), represents the most basic form of a connectome. It tells us which neurons are connected to which others, but reveals nothing about the strength of those connections, the types of neurotransmitters involved, or the dynamics of neuronal activity. While undoubtedly a monumental achievement that birthed the field of connectomics, the 1986 connectome provides only the most basic blueprint for anyone attempting to create a working emulation.

(On hidden complexity) Furthermore, the oft-cited "302 neurons" figure, while accurate, risks oversimplifying the complexity of the C. elegans nervous system. Indeed, this seemingly small network displays remarkable sophistication at the molecular level. C. elegans neurons are highly specialized and utilize a vast repertoire of molecular components for intracellular signaling and synaptic communication. In fact, signaling proteins constitute a staggering 20% of the worm's genome, and its synaptic machinery comprises roughly half the number of postsynaptic proteins found in mammals, with 288 identified postsynaptic density components compared to 570 in humans (Emes et al., 2008). The worm brain, constrained by its limited neuron count and fixed connectivity relies heavily on this molecular complexity to achieve its behavioral repertoire. This

includes performing several operations in a single cell using internal molecular circuits to perform functions. For example, a single sensory neuron, AFD, determines the worm's temperature preference by adding new proteins to its intracellular signaling network. Another neuron, AWCON, changes a behavioral response to suit the situation: when an odorant is present without food, AWCON's molecular receptors adapt and chemotaxis declines; however, when the same odorant is present with food, its receptors are sensitized, and chemotaxis increases. These competing responses are controlled by an intracellular mechanism that switches the connection between sensor and behavioral output to reverse the control of chemotactic turning behavior. Individual neurons in C. elegans can integrate diverse sensory inputs and modulate their responses based on context, often through intricate intracellular chemical signaling pathways. This strategy, while efficient in a compact nervous system, underscores a crucial point: understanding the worm's behavior requires far more than just knowing which neurons are connected.

(On previous attempts) Early emulation attempts, such as the Nemaload project led by David Dalrymple (circa 2011-2013) were severely hampered by the limitations of the neurotechnologies available at the time. Optogenetic perturbation, a crucial tool for manipulating neural activity, had only just been demonstrated in C. elegans in 2005. Comprehensive, large-scale neural recordings in the worm were, and to a large degree still are, in their infancy (Stiefel and Brooks, 2019) – partly due to the dynamic nature of the worm's nervous system which, with its constant motion and deformation, made tracking of individual neurons a challenge (Nguyen et al., 2017). This confluence of technological limitations—in neural recording, perturbation, tracking, and imaging—meant that the kind of comprehensive, dynamic data needed to truly understand and emulate the worm's nervous system simply wasn't available during Nemaload's time, and contributed to the development of new organizational structures – like Focused Research Organizations (FROs) – capable of tackling projects requiring such a high degree of systems integration.

(Other challenges) Moreover, early emulation efforts like Nemaload were further constrained by a lack of funding and resources. Nemaload received initial seed funding on the order of tens of thousands of dollars, followed by a larger investment in the hundreds of thousands. However, the estimated budget required to fully realize the project's goals – likely a significant underestimate, given the state of neurotechnology back – was at the very least an order of magnitude higher, and the necessary funding ultimately failed to materialize. This stark reality underscores a broader issue that continues to plague the field: a chronic lack of investment in connectomic-driven simulation work. Indeed, the current landscape of connectomic-driven simulation remains sparsely populated and under-resourced. Only a handful of groups worldwide are actively pursuing C. elegans emulation, often with limited personnel and funding. These include a small nonprofit organization relying on part-time volunteers, a few academic labs with limited manpower, and one postdoc leading a

biophysical simulation of the fruitfly.

(Box outro) Only now, with the advent of high-throughput connectomics, advanced imaging techniques, and increasingly sophisticated computational tools, are we beginning to amass the data necessary to make meaningful progress towards C. elegans emulation. Modern calcium imaging techniques allow us to observe neural activity across the entire worm brain in real-time, while new molecular tools help us understand the crucial intracellular signaling mechanisms. Combined with improved computational resources and better-organized research efforts we could be finally equipped to tackle the true complexity of even this 'simple' nervous system. The C. elegans story thus serves as both a cautionary tale about underestimating biological complexity and an optimistic reminder that with proper tools, funding, and organizational structures, progress in brain emulation is possible.

(Computational infrastructure / needs based on primitive extrapolations of the known numbers) 302 Neurons and approximately Y synapses add up to [] Bytes in simple models to [] Bytes in the most complex representations of simulation methods discussed above. The computational resources to run such a simulation span [] equivalent to X modern GPUs (H100).

OpenWorm

OpenWorm, launched in 2011, represents one of the most comprehensive attempts to create an integrative biological simulation of C. elegans, implementing a multi-scale biophysical approach (see Virtual Worm Project "worm body") that spans from ion channel dynamics to whole-organism behavior (Sarma et al., 2018).

(Data usage) The simulation architecture integrates multiple types of experimental data, though significant gaps remain. Connectome data from electron microscopy provides the basic network structure (White et al., 1986), while calcium imaging data informs neural dynamics. However, a major challenge is the limited availability of electrophysiological data - patch-clamp recordings exist for only a small subset of ion channels, necessitating the use of homology-based inference from other organisms for parameter estimation (Sarma et al., 2018).

(Architecture and Embodiment) The project's core simulation stack consists of several interconnected frameworks (see figure below), enabling simulation at multiple levels of

abstraction - from basic integrate-and-fire neurons to detailed multi-compartment models incorporating Hodgkin-Huxley dynamics (Gleeson et al., 2018). A critical limitation in the current implementation is the unidirectional flow of information from neural simulation to body mechanics, though work is ongoing to incorporate sensory feedback (Sarma et al., 2018).

Figure from (Sarma et al 2018)

PyOpenWorm for data management, ChannelWorm for ion channel modeling, c302 for neural simulation, and Sibernetic for body mechanics and environment interaction. The c302 framework, built on NeuroML and NEURON. Sibernetic employs predictive-corrective incompressible smoothed particle hydrodynamics (PCISPH) to simulate the worm's body and its interaction with fluid or gel environments (Palyanov et al., 2018). You should have at least 60 GB of free space on your machine and at least 2GB of RAM (Github page, 2024)

Figure 1. Overview of OpenWorm simulation stack. (*a*) A component diagram describing the relationships between inputs and outputs of sub-projects within OpenWorm. (*b*) A highly simplified schematic view of the system of equations executed in the combined c302/Sibernetic system.

(Validation) The project employs sophisticated validation frameworks, particularly SciUnit, to systematically compare model outputs against experimental data across multiple scales (Gerkin

et al., 2018). Behavioral validation focuses on comparing simulated movement patterns against real worm tracking data, while neural validation involves comparing activity patterns with calcium imaging recordings (Javer et al., 2018). The current implementation can demonstrate basic locomotion, though matching the full repertoire of C. elegans behaviors (including chemotaxis, thermotaxis, and learning) remains a future goal. These limitations reflect both computational challenges and gaps in biological understanding of how sensory information is processed to generate behavior.

From Github page

(outlook) The OpenWorm project makes its tools and simulations accessible through the Geppetto visualization platform (Cantarelli et al., 2018), enabling web-based exploration of models and data. This infrastructure, combined with the project's open-science approach, provides a foundation for community-driven refinement of whole-organism simulations, though significant challenges remain (Larson, interview 2021):

- Coordinating between different research groups with specialized expertise (in areas like movement tracking, calcium imaging, computational modeling etc.)
- Limited funding and institutional support for this kind of integrative work

- The need to bridge gaps between different scientific disciplines and methodologies in coordinating the diverse expertise required to advance the project.

Simeon ML Approaches

Recent approaches leveraging artificial neural networks (ANNs) offer a complementary data-driven strategy for modeling C. elegans neural dynamics, with particular emphasis on capturing the inherent predictability of neural activity patterns without requiring explicit biophysical modeling Simeon et al 2024. This represents a shift from traditional bottom-up approaches toward letting the underlying structure emerge from experimental data.

(Data usage) These approaches make extensive use of calcium imaging datasets, integrating recordings from multiple experimental sources spanning different behavioral contexts - from freely moving to immobilized worms, and from sleep to optogenetically stimulated states. The combined dataset represents neural activity from 284 worms. A key innovation is the standardization of data processing across sources, including z-scoring of calcium signals and temporal alignment, enabling the pooling of data despite varying experimental conditions.

(Architecture) The approach currently focuses on neural activity prediction without direct connection to behavioral outputs. The simulation framework employs multiple neural network architectures, incl. Long- Short Term Memory (LSTM) networks, Transformer networks, and Feedforward networks. All architectures share a common structural framework consisting of an embedding layer for neural state representation, a core processing module, and a readout layer for prediction.

(Validation) Model validation focuses on next-time-step prediction accuracy, using a teacher-student framework where the biological nervous system serves as the teacher and ANN models as students. A key methodological strength is the use of synthetic datasets with known underlying dynamics (including sinusoidal oscillators and random walks) to benchmark model performance. The approach employs a rigorous 50:50 temporal split for validation, where models are trained on the first half of neural recordings and tested on the second half, ensuring genuine predictive capability rather than mere memorization.

(Current capabilities and limitations) Current implementations demonstrate success in short-term prediction of neural activity patterns across up to 20 seconds (alignment across

20-30 timesteps of ~0.7s each), with recurrent models (LSTM and CTRNN) showing superior performance compared to other architectures. However, long-horizon predictive capabilities remain limited. It's worth noting that this represents prediction of autonomous neural activity without any sensory input or behavioral context. The models show consistent scaling properties across different experimental conditions, suggesting they capture fundamental aspects of neural dynamics. The CTRNN models showed the best scaling properties with a slope of -0.65, meaning that every time you double the amount of training data, you reduce the prediction error by about 2^0.65 ≈ 1.57 times. Expanding prediction from 20 to the full 180 timesteps would accordingly require a dataset ~40x larger ((x)^0.65 = 8), i.e. data from 10,000 worms.

BAAIWorm

BAAIWorm, introduced in 2024, represents a significant advancement in integrative biological simulation of C. elegans by implementing a closed-loop system bridging brain dynamics, body mechanics, and environmental interactions (Zhao et al 2024). The project builds upon OpenWorm's foundational contributions, particularly its cell model morphologies, synaptic dynamics, and 3D body representations, while introducing crucial new capabilities for real-time simulation and behavioral feedback.

(Data usage) The model integrates diverse experimental data, including ion channel dynamics, neural morphologies, electrophysiology, and whole-brain calcium imaging. A notable strength is the validation of single-neuron models against patch-clamp recordings for five representative neurons (sensory, inter-, command, and motor neurons), with parameters for other neurons derived through functional grouping. While this approach represents the most comprehensive integration of C. elegans data to date, the implementation acknowledges significant gaps in available data prior to 2023. The body-environment component utilizes detailed anatomical data to construct a biomechanical model comprising 3,341 tetrahedrons and 96 muscles.

(Architecture and Embodiment) The neural network model implements 136 neurons as multicompartmental models with sub-2μm compartments, incorporating 14 types of ion channels. Rather than enforcing strict neurotransmitter constraints, the model uses an optimization approach to determine synaptic properties that reproduce observed dynamics. The body-environment simulation employs simplified but efficient hydrodynamics, focusing

on thrust and drag forces while assuming neutrally buoyant conditions. This pragmatic compromise enables real-time simulation at 30 frames per second while maintaining behaviorally realistic movement patterns.

(Validation) The model demonstrates multi-scale validation, from single-neuron current-voltage characteristics to network-level correlation matrices (achieving 0.076 mean squared error against calcium imaging data) and behavioral reproduction of chemotaxis. While the current implementation focuses specifically on zigzag locomotion, synthetic perturbation experiments reveal important insights about neural circuit function - notably that the absence of neurites or synaptic/gap junctions disrupts global neural dynamics and impairs forward motion. A novel Target Body Reference Coordinate System enables precise quantification of movement patterns, providing a stable framework for comparing simulated and biological behavior.

(Outlook) While BAAIWorm represents a significant advance in integrating different scales of biological simulation, several challenges remain, including expanding to the full 302-neuron network and incorporating additional behaviors beyond chemotaxis. The platform's modular architecture and open-source implementation provide a foundation for community-driven refinement, though computational requirements may limit accessibility. Perhaps most importantly, BAAIWorm demonstrates a viable approach to balancing biological detail with computational feasibility, offering insights for future efforts in whole-organism simulation.

Figure: Video showing the simulated worm moving towards simulated chemicals. (from here)

Figure from paper

Fig. 2 | Construction of biophysically detailed neural network model of *C. elegans*. **a**, Experimental data collection to constrain models. These data include neural morphologies, ion channel models, electrophysiological recordings of single neurons, connectome, connection models and neural network activities. **b**, The construction of multicompartmental neuron models and connection models (synapses and gap junctions). **c**, The biophysically detailed *C. elegans* neural network model without functional neural activities. **d**, Optimization of the biophysically detailed *C. elegans* neural network model to achieve realistic network dynamics. Neurons are color-coded to represent membrane potential.

Drosophila

Drosophila brain simulations have been profoundly shaped by the relative abundance of connectomics data, from earlier partial reconstructions in FlyCircuit to the recent release of a full adult connectome. This detailed structural knowledge has enabled increasingly comprehensive models, from circuit-specific implementations to whole-brain simulations attempting to capture system-wide dynamics.

Huang et al., 2019 and Higuchi et al., 2022

Early attempts at whole-brain Drosophila simulations were constrained by the absence of a fully reconstructed connectome. These efforts instead leveraged the FlyCircuit database (Chiang et al., 2011), which provided a collection of high-resolution 3D confocal microscopy images of thousands of neurons, each genetically labeled with GFP in different fly brains and then registered to a common template, but without direct synaptic connectivity information. Huang et al. pioneered methods to infer whole-brain connectivity from this partial morphological dataset – containing approximately 20,000 neurons in 2019, or roughly 14% of the 140,000 neurons in the Drosophila brain (Huang et al., 2019). Their approach used machine learning to predict input/output regions of each neuron, combined with spatial proximity analysis to predict likely connections. Higuchi et al. worked with a similar subset of FlyCircuit neurons (18,728 after filtering out damaged and larval neurons), using related methods to determine neuron polarity but developing more sophisticated approaches for estimating connectivity based on spatial proximity with specific distance thresholds informed by electron microscopy studies (Higuchi et al., 2022).

The two efforts chose different modeling frameworks. Huang et al. utilized simplified leaky integrate-and-fire neurons with conductance-based synapses. In contrast, Higuchi et al. leveraged the Fugaku supercomputer to implement biophysically detailed multi-compartment Hodgkin-Huxley dynamics, where each neuron was segmented into cylindrical compartments with local cable equations governing voltage propagation. Their model achieved near real-time simulation despite the increased computational complexity. Both models derived membrane parameters from neuronal morphology, but Higuchi's implementation included more sophisticated estimates based on detailed geometrical features.

The two groups also took different approaches to validating their models. Huang et al. focused on comparing network-level dynamics against randomized control networks with the same number of neurons but random connections, demonstrating their reconstructed network exhibited more biologically plausible stability and firing patterns. Higuchi et al. validated their model by comparing simulated firing rates against experimental measurements from real Drosophila neurons, and by demonstrating the network could reproduce known circuit-level behaviors in olfactory learning tasks.

Lappalainen et al., 2024

Another influential example of Drosophila modeling leveraging partial connectomics data came from Lappalainen et al., who developed a "differentiable mechanistic network" (DMN) constrained by anatomical connectivity (Lappalainen et al., 2024). Their approach combined two electron microscopy datasets (702 neurons from FIB-25 and 1,099 from FIB-19) spanning different regions of the optic lobe. This connectivity data was complemented by transcriptomics to determine synaptic signs based on neurotransmitter expression. Since the fly's visual system is organized in repeating columns of similar neural circuits, they used their detailed reconstructions of a small region to build a larger model spanning approximately 45,000 neurons across 721 columns of the central visual field. The team trained their model on synthetic visual inputs, converting frames from the Sintel film database (Butler et al., 2012) into hexagonal arrays of photoreceptor activations that matched the fly's eye structure. Type-specific parameters like membrane time constants and resting potentials were optimized for motion detection - specifically, to predict the direction and speed of movement for each point in a visual scene.

Each neuron was treated as a leaky, non-spiking node, with graded synapses encoding excitatory or inhibitory interactions based on known transmitter identities. A small number of free parameters captured type-level properties. To translate the network's neural activity into motion predictions, they used a simple convolutional neural network that took the activity of a subset of neurons as input and produced estimates of movement direction and speed. Model validation involved comparing these motion predictions against ground truth from the Sintel database, alongside testing whether the model reproduced known properties of the fly visual system (e.g., ON/OFF selectivity, T4/T5 direction tuning) established by prior experimental studies. Although training was successful in reproducing core visual computations, ablation

analyses revealed strong dependence on correct synaptic signs and connectome structure, underscoring the importance of biologically grounded constraints.

Shiu et al.

The release of the Flywire reconstruction of the adult Drosophila brain connectome (Dorkenwald et al., 2024) enabled a new generation of simulations characterized by both whole-brain coverage and connectome-derived structure constraints. Shiu et al. developed one of the first comprehensive models incorporating this data, working with over 127,400 proofread neurons and their 50-plus million synaptic connections (Shiu et al., 2024). To assign neurotransmitter identities, they leveraged prior large-scale predictions (Eckstein et al., 2024), broadly classifying neurons as excitatory (primarily cholinergic) or inhibitory (GABAergic or glutamatergic). Dopaminergic, octopaminergic, and serotonergic neurons were also incorporated and treated as excitatory. Connection weights were derived directly from the Flywire connectome, with sign determined by the assigned neurotransmitter identity. The model required fitting only a single free parameter - the global synaptic weight scale (Wsyn). This parameter, a global scaling factor, determined how strongly each synapse influenced postsynaptic membrane potential, regardless of the specific neurons involved. It was calibrated using known feeding circuit dynamics, specifically tuned so that 100 Hz activation of sugar-sensing gustatory receptor neurons produced approximately 80% of maximal firing in motor neuron 9 (MN9), a key neuron controlling extension of the fly's proboscis (feeding appendage). This calibration point was chosen based on extensive prior experimental characterization of the sugar sensing to feeding initiation pathway.

The model employed a leaky integrate-and-fire (LIF) framework with α-synapse dynamics, incorporating synaptic conductance, membrane resistance, and time constants derived from previous Drosophila modeling and electrophysiological studies. On the whole, the model made several simplifying assumptions: neurons had zero basal firing rates, gap junctions were not available and thus excluded, and neuromodulatory effects beyond basic excitation/inhibition were not incorporated.

Despite those simplifications, validation efforts yielded encouraging results. To validate their model, Shiu et al. primarily focused on simulating two well-characterized circuits: the feeding circuit and the antennal grooming circuit. For the feeding circuit, they computationally

activated subsets of gustatory receptor neurons (sugar, water, bitter, and Ir94e-expressing) and analyzed the resulting patterns of network activity. Similarly, they simulated the antennal grooming circuit by activating mechanosensory neurons in the Johnston's organ, a sensory structure in the antenna that detects antennal movements. The authors employed three primary approaches to validate these predictions: direct comparison of computationally predicted neural activity with experimental calcium imaging data, in silico silencing experiments to assess the necessity of specific neurons (validated against genetic silencing experiments in real flies), and optogenetic activation studies testing whether specific neurons were sufficient to elicit the behaviors predicted by the model (without embodiment). Through this multi-faceted validation strategy, they demonstrated their model could accurately recapitulate known feeding and grooming behaviors, achieving 91% accuracy across 164 experimental predictions and generating novel insights like the inhibitory role of Ir94e neurons in feeding.

Cowley et al., 2024

Unlike the connectome-driven approaches described above, Cowley et al. developed their model primarily through behavioral and functional constraints (Cowley et al., 2024).. Their approach centered on understanding how specific neuron types contribute to behavior by systematically silencing neurons and incorporating these perturbations into model training. For model fitting, they collected behavioral data from 459 male-female fly pairs during courtship interactions. They recorded six behavioral variables from the male: three movement parameters (forward velocity, lateral velocity, and angular velocity) and three measures of song production (sine song, fast pulse song, and slow pulse song). In each experimental condition, they genetically silenced one of 23 different visual projection neuron (LC) types that form a bottleneck between the optic lobe and central brain. Their model consisted of three components: a convolutional network processing the male's reconstructed visual experience, a bottleneck layer of 23 units (each representing one LC type), and a decision network producing behavioral outputs. The model processed sequences of 10 frames (~300ms) of visual input to predict behavior, and its training involved "knockout training": when training on data from flies with a silenced LC type, Cowley et al. set the corresponding model unit's activity to zero, forcing the network to learn how each LC type contributes to behavior. To validate their model, they performed two-photon calcium imaging in head-fixed males from five LC types, testing responses to both artificial and naturalistic visual stimuli. Despite being

trained only on behavioral data, their model achieved 35% correlation with neural responses. The model's prediction that LC types work in combination rather than as independent channels was further supported by analysis of the FlyWire connectome, which revealed shared inputs and outputs among LC types.

NeuroMechFly and Janelia et al.

Parallel to these advances in brain simulation, there has also been significant progress in developing detailed embodied simulations of Drosophila. While these models currently employ relatively simple neural controllers compared to the simulations discussed above, they represent an important complementary approach that could eventually enable the study of a broader range of fly behaviors when combined with more sophisticated neural models. Two notable efforts in this direction are the NeuroMechFly project and the recent work by Vaxenburg et al. The NeuroMechFly team developed a morphologically accurate model derived from X-ray microtomography data with 65 body segments and 122 degrees of freedom – where each DoF represents an independent type of movement or rotation possible at a joint. The model initially focused on walking and grooming behaviors (Lobato-Rios et al., 2022). Their first version learned to produce stable walking patterns matching those seen in real flies (video), including the typical three-legged walking pattern where legs move in alternating groups of three. Their 2024 update expanded the model's capabilities to include vision and smell, demonstrating more complex behaviors like following scent trails while avoiding obstacles in their path (Wang-Chen et al., 2024). Notably, this updated version integrated the connectome-constrained model developed by Lappalainen et al. to simulate visual processing during a fly-following task. Vaxenburg et al. took a different approach, building their model from high-resolution confocal microscopy data with 67 body segments and 102 degrees of freedom, along with sophisticated physics modeling including fluid dynamics for flight and special features that allowed the simulated fly to stick to surfaces like real flies do (Vaxenburg et al., 2024). Through reinforcement and imitation learning, their ANN-driven model successfully replicated both walking and flight behaviors from real fly trajectories, including complex maneuvers like rapid turns and sudden changes in direction. Their model also demonstrated behaviors such as maintaining altitude over uneven terrain and navigating through winding trenches without collision.

Larval Zebrafish

The landscape of larval zebrafish simulation efforts is distinctly shaped by three key factors: the current absence of a fully reconstructed connectome; its relative novelty as a brain simulation model organism in computational neuroscience compared to C. elegans; and the optical transparency of its brain, making whole-brain calcium imaging data readily accessible. These characteristics have led to simulation approaches that are generally not whole-brain, not connectome-constrained, and revolve around functional data as the key to model fitting and validation.

Vishwanathan et al.

Vishwanathan et al. implemented a highly simplified linear rate model as a proof of concept for connectome-constrained circuit simulation in larval zebrafish. It was based on a reconstructed 0.0024 mm^3 brainstem volume (approximately 2.4% of the total brain volume) containing circuits involved in oculomotor and velocity-to-position integration (Vishwanathan et al., 2024). Based on the identified ~3,000 neurons, ~75,000 synapses, they derived a directed graph weighted by synaptic counts between neurons, with weights normalized by the total synaptic input to each postsynaptic neuron and with prior physiological studies of neurotransmitter identities and anatomical mapping of circuit components used to further constrain the model.

Model validation leveraged previously collected two-photon calcium imaging data from 20 different larval zebrafish, combined with simultaneous eye position recordings. The validation focused on two key aspects of oculomotor integration: how neuron firing rates correlated with eye position during fixations, and how neural activity changed following rapid eye movements. For each neuron population, the authors compared how strongly neurons respond to eye position changes in the model versus experimental measurements from calcium imaging across 20 different zebrafish - the model, despite being based on a single specimen's connectivity, correctly predicted the characteristic distribution of position sensitivities for each population.

Anonymous et al.

(Data usage) Anonymous et al. developed a black-box machine learning model trained on approximately two hours of 1 Hz calcium recordings from the ZAPBench dataset (Anonymous authors, 2024), which captures the activity of approximately 70,000 neurons

across the whole brain (2048 x 1152 x 72 voxels at 406 nm x 406 nm x 4 µm resolution, likely 70-80% of all neurons of the organism) from a single head-fixed but tail-free larval zebrafish during nine different behavioral tasks. The recordings were obtained using light-sheet microscopy while the fish was exposed to various visual stimuli, including forward-moving gratings to test gain adaptation, random dot patterns for decision-making, alternating light/dark flashes for startle responses, asymmetric illumination for phototaxis, and various other motion patterns to probe turning behavior and positional homeostasis. Throughout the experiments, the fish's tail movements were monitored via electrical recordings from motor nerves, allowing real-time coupling between the fish's attempted swimming behavior and the visual stimuli presented (Anonymous authors, 2024).

(Architecture) Unlike traditional approaches that first segment individual neurons and extract their activity traces, this method worked directly with the raw volumetric video data, aiming to predict future frames of brain-wide activity. The authors implemented a 4D UNet architecture operating on three spatial dimensions plus time, trained end-to-end to minimize the mean absolute error between predicted and actual (voxel-based) calcium traces. The model was trained on approximately 1.4 hours of data (70% of the full 2-hour recording) spanning 8 different behavioral conditions, with performance evaluated on held-out test segments from these conditions as well as a completely held-out ninth condition. Attempts to improve performance through pre-training on data from two other specimens proved unsuccessful - though this single result on a new benchmark with few baselines provides limited insight into the general challenge of transfer learning across individuals.

(Validation) When validated against held-out recordings, the model achieved higher prediction accuracy for short temporal contexts, though showed comparable performance to trace-based approaches when using longer temporal contexts. On completely held-out experimental conditions, the model demonstrated better generalization for one-step-ahead predictions but not for longer forecast horizons. Successful prediction, at least when defined in terms of mean absolute error, thus remains a challenge for both trace-based and video-based models, especially with longer temporal contexts. (Outlook) While this work demonstrates the feasibility of purely ML-based approaches to neural activity prediction in Zebrafish larvae, the main challenge remains sample efficiency - as these models lack biological priors constraining their predictions, they require substantially more data to learn effectively. Future work will likely

focus on improving sample efficiency through better architectures and finding ways to successfully leverage data across individuals through cross-specimen pre-training. The connectome for this exact fish will be coming relatively soon, thus enabling a variety of approaches that incorporate serious biological priors.

Liu et al.

A third distinct approach to larval zebrafish simulation focuses on embodied models bridging sensory processing, neural control, and biomechanics to reproduce behavior. A notable example is simZFish, developed by Liu et al. on the basis of their previous behavioral and calcium imaging recordings of the optomotor response - a behavior where fish adjust their swimming to maintain position when their entire visual environment appears to move around them (Liu et al., 2024; Naumann et al., 2016). The model was fit using data from their 2016 work, where they identified distinct neural types in the pretectal circuit by analyzing calcium imaging recordings from 3,070 neurons across 12 fish, characterizing their firing patterns during visual stimulation and establishing their functional connectivity through analysis of concurrent behavioral recordings from 38 fish.

(Architecture & Embodiment) This characterization was used to initialize a simplified, biologically-inspired rate-coding network. However, the model's key contribution lies not in its neural simulation but in its detailed biomechanical implementation, comprising seven body segments with realistic hydrodynamics and a virtual visual system with two cameras mimicking the fish's eyes. (Validation) The model underwent comprehensive validation through multiple approaches: behavioral analysis comparing simulated and real fish responses to visual stimuli, comparison of artificial neural activity patterns to calcium imaging recordings, and finally, implementation in a physical robot tested in both controlled and natural environments. Both simulation and robot (see figure below) demonstrated the ability to maintain position in moving water through visual input alone.

OMR
0s
OMR off
0s
Motors off
0s

Mouse

The Blue Brain Project

(Data Usage and Architecture) Having been started before large-scale connectomic datasets were available, the blue brain project developed various tools to integrate disparate datasets describing cell types and connectivity in rodent cortex. Cortical anatomy of their largest model release (Reimann et al., 2024) was based on a three-dimensional cell atlas, in which cell bodies of 60 morphologically distinct cell types were placed based on experimentally reported densities. From there, detailed multi-compartment models of cells were expanded based on known shape constraints and populated with ion channels based on parameter-tuning to recreate *in vitro* recordings. Connectivity is inferred based on biological rules and validated by comparing statistics of resulting synaptic densities with those reported experimentally. Synapses are modelled on a detailed level, including a pool of available neurotransmitter vesicles and short-term plasticity. In total, they reconstructed 36mm^3 of rat somatosensory cortex, comprising 4.2 million neurons with 14.2 billion synapses between them. For computations they had access to the Blue Brain 5 supercomputer, providing 0.8 TFlops of computational power.

(Validation) Electrical properties of single neurons were validated by optimising ion channel densities in different neuronal compartments to give rise to firing properties, action potential waveforms and passive properties observed in vivo (Reva et al., 2023). Synaptic parameters were fitted in a similar way (Ecker et al., 2020). The size of the model allowed modelling not only of local connections within a cortical column, but also mid-range connections between close brain regions (Isbister et al., 2024), and spontaneous activity was comparable to that observed *in vivo*. Long-range connections providing e.g. sensory input still had to be approximated. The movement of whiskers, as an example for external sensory input, was modelled by directly injecting current into the soma of neurons projecting from the thalamus to the cortex, mimicking the flow of information in real brains. This produced activity congruent with that observed *in vivo* on the millisecond scale in model parameterisations representing awake and anesthetized animals. Additionally, by artificially hyperpolarizing selected neurons in their simulation, they could reproduce results produced by optogenetically inactivating neurons in animals.

Image from [Isbister et al., 2024]
Overview over the blue brain project's largest cortical simulation

Billeh et al., 2020

(Architecture) A similar approach to the Blue Brain Project was taken by the Allen Institute for Brain Science (Billeh et al., 2020). By estimating cell densities and types from anatomical data, they modeled a 400μm column of visual cortex containing ~52,000 neurons with high biological fidelity, surrounded by 179,000 point-neurons to avoid boundary artifacts. Connection parameters were inferred from various physiological datasets and connections

optimised in a layer-wise manner to produce firing dynamics that match *in vivo* recordings. Input was modelled via a module that mimics thalamic input to the visual cortex. This module was composed of spatial filters following experimentally reported distributions, which were then connected to cortical neurons to give rise to established tunings. Connection strengths were set to match experimentally reported current strengths. In total, this allows encoding arbitrary spatiotemporal stimuli via simulated thalamocortical spike trains.

(Validation) Connectivity was verified by comparing the activity of simulated neurons in response to drifting gratings with *in vivo* neuropixel recordings. Activity was compared via average firing rates, direction selectivity and orientation selectivity for different neuron classes and cortical layers. A later study (Rimehaug et al., 2023) found that model responses on the level of current source density were inconsistent with results from neuropixel recordings, and improved it by adjusting connection weights and including feedback connections.

Wang et al

(Data Usage & Architecture) Wang et al. (2023) based their work on cortical activity rather than on explicit connectivity information: Utilising a dataset containing 900 minutes of two-photon recordings of calcium traces in 67000 visual cortex neurons in response to natural movies, as well as recordings of behavioural variables such as pupil position and movement speed, they trained a recurrently connected deep neural network to predict the activity of individual neurons. The full network consists of a perspective network, which uses ray-tracing to infer the retinal activation of the mouse based on pupil position and stimulus, the modulation network, an LSTM network that encodes behavioural variables, a recurrent foundation core that captures abstract aspects of brain processing, and a readout network, which maps activity of the core network to individual neurons. By fixing the weights of the foundation core and only retraining encoding and decoding networks, the researchers were able to combine data from 8 mice and achieve higher accuracy than with networks trained end-to-end for each individual mouse.

(Validation) Quality of the network was validated by predicting the activity of neurons to novel visual stimuli and calculating the normalised cross-correlation with held-out *in vivo* recordings. Additionally, the properties of *in silico* neurons were compared to those of *in vivo* neurons,

showing that they had developed the same parametric tuning properties concerning orientation and spatial position. When training a network containing the foundation core to predict the activity of the Microns cortical mm^3, *in silico* neurons exhibited functional specialisation and retinotopy, both well-characterised aspects of cortical functioning.

Image from Wang et al. (2023)
Setup of foundation model training paradigm

Humans

Unlike organisms like Drosophila or larval zebrafish, computational neuroscience of the human brain operates under severe data constraints. There is no synapse-level connectome, and functional recordings either cover extremely limited circuits (in the case of invasive recordings) or provide only indirect measures of neural activity at low spatial and temporal resolution (in the case of non-invasive methods like fMRI). This fundamental limitation has shaped a landscape dominated by feasibility studies - efforts focused primarily on demonstrating the possibility of simulating human brain-scale networks on current supercomputing infrastructure, rather than attempting to replicate specific circuits or behaviors with high biological fidelity.

Yamazaki et al., 2021

Yamazaki and colleagues developed one of the first human-scale simulations of the cerebellum using their MONET simulator on Japan's K supercomputer, capable of 11.3 PFLOPS (Yamazaki et al., 2021). (Data Used) Their model leveraged anatomical studies providing cerebellar layer thicknesses and cell density measurements across different regions. This structural data informed both the spatial organization and the number of neurons of each type in their model.

(Model Construction & Architecture) From this anatomical data, they constructed a network of 68 billion neurons and 5.4 trillion synapses. Neurons were modeled as conductance-based leaky integrate-and-fire units with α-function synapses implementing four receptor types (AMPA, NMDA, GABAA, GABAB). Connection patterns between neurons were defined using two-dimensional Gaussian distributions, with parameters like connection probabilities and spatial extents derived from anatomical studies.

(Validation) Model validation occurred in two stages. First, they examined resting state activity, comparing baseline firing rates across different cell types with experimental data. Second, they tested the model's ability to reproduce the optokinetic response (OKR), a reflexive eye movement controlled by the cerebellum. For OKR, they found Purkinje cells modulated their firing rates between 50-80 Hz, within the range observed across various animal studies (20-100 Hz). Running on 82,944 nodes of the K computer, the simulation achieved speeds 578 times slower than real-time.

Lu et al., 2023

Lu and colleagues developed the "Digital Twin Brain" (DTB) platform, implementing a whole-brain simulation of 86 billion neurons and 47.8 trillion synapses on a GPU-based supercomputing cluster (Lu et al., 2023).

(Data Used) Their model's structure was informed by three types of macroscopic brain imaging data: structural MRI provided regional neuron densities at 3x3x3mm^3 voxel resolution, diffusion tensor imaging (DTI) determined voxel-to-voxel connection probabilities, and PET data established what proportion of connections remained local vs projecting to other regions (varying from ~29% external connections in cortex to ~13% in cerebellum). Of all possible connections between voxels, only 0.72% were realized, reflecting the brain's sparse connectivity.

(Model Fitting) They developed a "hierarchical mesoscopic data assimilation" (HMDA) approach to tune model parameters. This involved first training a smaller network (0.2 billion neurons) to estimate hyperparameters for synaptic conductances, then using these to initialize the full model. Parameters were iteratively refined by comparing simulated BOLD signals to real fMRI data during a visual evaluation task.

(Model Architecture) Each neuron was modeled as a conductance-based LIF unit with four receptor types (AMPA, NMDA, GABAa, GABAb). Average input synapses per neuron were set to 1000 for cortical/subcortical regions and 100 for brainstem/cerebellum. Long-range connections were restricted to excitatory neurons only. To manage computational demands on their GPU cluster (where each node contained four GPUs with 16GB memory each), they developed a partitioning algorithm that balanced computational load and minimized inter-GPU communication.

(Validation & Results) The fitted model achieved correlations above 0.98 with experimental BOLD signals in input regions (with a 2-timepoint lag) and 0.75 across the cortex. When trained to predict participants' ratings of emotional images, the model achieved significant correlation with actual ratings ($r=0.655$, $p=0.006$). Running at different firing rates (7Hz,

15Hz, and 30Hz), the simulation achieved speeds between 65-118.8 times slower than real-time.

Miscellaneous Organisms

None yet

Funders

Brain initiatives + funding

Funding for projects in the broader ecosystem of brain emulations. Nearly all initiatives began in the past ~10y. This points to the recent advances that enabled large-scale brain mapping, such as imaging, sequencing, and computing. Most active projects have been ongoing for 5+ years, underlining the long timescales involved. Methodological novelty and interdisciplinary integration are common differentiators. Several aim for more detailed mapping or broader data integration.

Early projects focused heavily on mouse models but there is a transition towards human studies now.

Figure: Total funding in neuroscience in the past 10 years. Of these 12.8B, approximately 20% directly contributed to any of the above mentioned methods for brain emulation efforts, the remainder being focused on topics like brain BCIs, and XXX (Brain Initiatives T4 v3.xlsx) Connectomics and brain simulations have not received outrageous amounts of money. By our estimates, academic and philanthropic brain initiatives with the broader scope of high resolution brain data acquisition have received low billions of dollars in funding cumulatively over the past 20y, somewhere in the range of $100-500M globally. The effective purchasing power is probably even lower given academia overheads for universities.

Government

(what has been done for the field) Advancing neurology and neuroscience has been part of research agendas in many countries. Neuroscience initiatives by governments globally provided roughly 8B Dollars in funding over the past decade. (Brain Initiatives T4 v3.xlsx). In chronological order these include:

project name	start year	end year	Neuroscience funding ($M)	funding within BRAIN ($M)	status	ref
Canada Brain Research Fund & Brain Canada Foundation	1998	n/a	280.00		Active	https://braincanada.ca/about/what-we-do/
UK Biobank Brain Imaging	2004	n/a	39.60		Active	https://www.ukbiobank.ac.uk/media/wgsfd0gg/uk-biobank-funding-details-summary.pdf (counting imaging only)
Synapse (DARPA)	2008	2016	100.00		Concluded	https://www.nextbigfuture.com/2012/06/darpa-synapse-project-status.html
Human Connectome Project	2009	2014	38.50		Concluded	https://journals.sagepub.com/doi/10.2310/JIM.0b013e3182025955
Child Mind Institute	2009	n/a	55.00		Active	at least 55M: https://childmind.org/blog/child-mind-institute-receives-55-million-stavros-niarchos-foundation-grant-for-global-childrens-mental-health/
Brain Research Through Advancing Innovative Neurotechnologies (BRAIN) Initiative	2013	n/a	4,000.00		Active	https://braininitiative.nih.gov/funding/understanding-brain-initiative-budget
BRAIN Initiative Cell Atlas Network (BICAN)				500		
BRAIN Initiative Cell Census Network (BICCN)				375		https://www.nature.com/articles/d41586-023-03954-y
BRAIN Initiative Connectivity Across Scales (BRAIN CONNECTS)				150		https://www.ninds.nih.gov/news-events/news/highlights-announcements/nih-brain-initiative-launches-projects-develop-innovative-technologies-map-brain-incredible-detail
Machine Intelligence from Cortical Networks (MICrONS)				100		
Human Brain Project (HBP)	2013	2023	607.00		Concluded	https://www.humanbrainproject.eu/en/about-hbp/human-brain-project-ebrains/
Blue Brain Project	2013	2024	242.00		Active	https://www.epfl.ch/research/domains/bluebrain/frequently_asked_questions/ (no. 8, not counting HBP-sourced funding)
Neuropixels 1.0	2013	2017	5.50		Concluded	https://www.hhmi.org/news/new-silicon-probes-record-activity-hundreds-neurons-simultaneously
Brain Mapping by Integrated Neurotechnologies for Disease Studies (Brain/MINDS) project	2014	n/a	350.00		Active	https://www.riken.jp/en/news_pubs/research_news/rr/20211223_1/index.html
Adolescent Brain Development (ABCD) study	2016	n/a	350.00		Active	calculated based on NIH reporter data (sourced from https://reporter.nih.gov/search/BACA3Kafx0Cc0ByVH3VkkA/projects?sort_field=fiscal_year&sort_order=desc#)
International Brain Laboratory (IBL)	2017	n/a	20.00		Active	https://en.wikipedia.org/wiki/International_Brain_Laboratory.
Courtois NeuroMod	2018	n/a	4.50		Active	https://docs.cneuromod.ca/en/2020-alpha/AUTHORS.html
Natural Scenes Dataset	2018	2023	0.40		Concluded	https://www.nsf.gov/awardsearch/showAward?AWD_ID=1822929&HistoricalAwards=false
International Center for Primate Brain Research (ICPBR)	2020	n/a	106.00		Active	https://www.nature.com/articles/d41586-019-00292-w
China Brain Project (CBP)	2021	n/a	446.00		Active	https://www.nature.com/articles/d41586-023-03954-y, https://archive.ph/fTyVn, but https://www.sciencedirect.com/science/article/abs/pii/S2095927323001214?via=ihub has 3.2B RMB = $460M, https://www.scmp.com/news/china/science/article/3164585/rare-public-row-erupts-over-funding-us16-billion-china-brain has $16B (???), https://www.nature.com/articles/d41586-024-03044-7 has 1.66B (???). gotta check the grant directly (2021.12-2026.12)
Precision Neurotechnologies	2024	n/a	88.00		Active	https://www.aria.org.uk/precision-neurotechnologies/
Korea Brain Initiative	n/a	n/a	657.00		Proposed	https://shs.cairn.info/journal-revue-internationale-de-politique-comparee-2022-1-page-151?lang=en#re9nc9
Australian Brain Initiative	n/a	n/a	500.00		Proposed	https://www.science.org.au/supporting-science/other-initiatives/ausbrain/why-australia-needs-brain-initiative

As a rough approximation, governments globally seem to spend on the order of 1-2B dollars globally on neuroscience related, but not targeted, at brain emulation. Additional investments in the context of neurodegenerative diseases, funding of universities, publicly accessible

computer infrastructure etc. indirectly contribute animal models, imaging technologies and basic scientific understanding of the brain.

Typically, neuroscience initiatives distribute sums broadly across institutions and scientists. The Human Brain Project for instance allocated ~600M EUR over 10y amongst ~500 Scientists at 155 institutions (HBP Final Report, 2024). The US BRAIN Connects project awards 150M dollars over 5 years amongst 40 universities (NIH, 2024). So while funding amounts are large, most individual investigators do not receive more than six figure amounts in yearly funding.

(Given their interests, capabilities and constraints, What drives this stakeholder?) Government decisions are motivated by investments that enable their societies to thrive. The US NIH's mission page, globally the largest funder of brain emulation related research, states "NIH provides leadership and direction to programs designed to improve the health of the Nation" and ensuring "high return on investment for U.S. taxpayers" (NIH, 2024, NIH).
Such scientific investments not only benefit via health, but also come with larger economic benefits bolstering the nation's relative technological capabilities, leadership and eventually economic output in forms of companies and jobs, including high-skill immigration. In several instances even national security applications exist, as the involvement of DARPA various life science projects, including the BRAIN initiative indicates (Whitehouse, 2013).

(Motivate this by providing non-neuro examples.) In this context it is helpful to compare the current pre-paradigmatic state of brain emulation to the advent of the Human Brain Project in the late 1980. What likely was a herculean effort of lobbying and convincing work by the scientific community, the yearly funding more than doubled (fact check) in the early 1990 with the human genome project. Only fractions of genomes genome had been sequenced at the nucleotide level when the human genome project was conceived. The first draft of the mouse genome wasn't published until 2002, 12 years after the human genome was started and mostly due to significant progress in the Human genome project (ref). In 13 years ~92-93% of the human genome was sequenced and the project was declared a success (Tripp and Grueber, 2011). It did not provide immediate health care benefits, nor did it "solve" genetic diseases. But it was an indispensable impulse for a whole ecosystem far beyond genetics. The Human Genome Project spent $8B (inflation adjusted to 2025) and took 13 years (Tripp and Grueber,

2011). Impressively, the US economy saw a return of $1,220B (a >140x return) on this amount spent in conjunction with the creation of over 300,000 additional jobs (Tripp and Grueber, 2011, ref, ref)— not counting the new era of genomics led by CRISPR was ushered in. The number of approved drugs with known protein targets rose from less than 50% before 2001 to nearly 100% for drugs licensed in the US after the HGP (Gates et al., 2021).

This is just one of many instances where scientific advancement generated lasting economic benefits. The Appollo program cost over $300B (inflation adjusted) (Dreier, 2022) and took 8 years to put a human on the moon (Loff, 2015). It is assumed to have a 7-8x return on investment (ref). Similarly, the development of DARPA-net as a predecessor of the Internet catalysed a trillion dollar internet economy. The list of government projects that surpass yearly neuroscientific investments in vast Funding - Brain initiatives, other projects

To many it might seems that the current investments into neuroscience haven't been that fruitful and recently budgets for major initiatives were cut (Science, 2024). Importantly, in-depth analyses of the societal benefits of governmental neuroscience investment have not been available at the point of writing this report, but the tools and capabilities developed so far very likely have made major contributions in healthcare, job-markets and tax-returns. The Human Brain Project's final report for instance states "92 filed patents and 12 spin-off companies" and "Substantial progress was made in bringing neuroscience significantly closer to new clinical and industrial applications." (HBP Final Report, 2024). It also should be expected that future direct, indirect and induced effects on society could be substantial and warrant a closer analysis. And while the reported returns of the human genome project are impressive, it is worth mentioning that the 40 year period prior to the human genome project starting with the discovery of DNA did not have such returns, but received substantial funding nevertheless. Potentially neuroscience is now in a similar position with 40years since the publication of the C. elegans connectome and will see most of its positive impacts in the upcoming decades.

Inspired by the application fields outlined by Tripp and Grueber, 2011 for the HGP (human and veterinary health, argiculture & food, industrial biotech, environment and forensics), the following list aims to provide readers with a rough overview of such effects frequently motivating research grants in the literature, but narrowed down to technologies discussed in this report. While necessarily incomplete and out of scope for this report, it gestures at the

value of such economic analyses. If a government were to invest as heavily and as targeted as the Human Genome Project on major brain emulation milestones, favorable immediate (e.g. job creation) and long-term returns on investments seem plausible.

Neurodynamics:

- **Invasive and non-invasive computer interfaces in health care** will benefit very directly from substantial advances in neurodynamics. Improving scale and quality of neuronal activity data acquisition across scales and organisms likely benevits invasive and non-invasive brain computer interfaces and contributes to substantially better input / output functions, On average, X % of a nation's population has disabilities that could be overcome with better BCIs. (ref)
- **Diagnostic applications:** advanced neuronal recording capabilities could enables more potent mental health diagnostics. Trained of vastly more and better data, real-time non-invasive brain state detection at low costs might become feasible both in a clinical "vital sign" setting as well as in consumer devices that could help the early detection and mitigation of brain states that lead to costly outcomes such as burn-out, depression, etc. [Markets for health devices like the apple watch, Aura ring, etc. indicate a substantial interest.] [say something about the biofeedback market]
- **Non-invasive Consumer applications:** Multi-billion dollar investments by organizations like Apple and Meta in virtual reality devices are ongoing. Similarly, virtual reality gaming markets are steadily increasing. The market for BCIs is still very nascent ($2.1bn in 2022, ~$6bn in 2028; Precedence Research, 2023), but with the latest developments in VR and AR devices ($25bn in 2022 to ~$60bn in 2028; Statista Inc., 2024) we will soon see the first applications beyond the medical field (Blankertz et al., 2010, Frontiers, 2022), especially an increase in personal and recreational devices.

Connectomics:

- **In-silico platforms:** Efforts that advance "ultra-high resolution connectomes", i.e. exploring the frontier of rich molecularly annotated connectomes and neuromodulators, might aid as in-silico experimentation platforms for brain related drugs. The level of detail of such simulations will be limited to small circuits or brain areas due to their data acquisition and computational demands, but this might be sufficient for pharmaceutical models. The global market for central nervous system

(CNS) treatment was $89bn in 2021 and may grow to ~$170bn by 2028 (Fortune Business Insights, 2022) and there is a huge interest in moving away from animal models in drug development (ref).

Computational Neuroscience:

- **Comparative Intelligence Studies & Micro AI:** While we do not fully understand the inner workings of networks underlying LLMs like ChatGPT we have both access to their structure and function, allowing us to run experiments and test how exactly intelligent behavior comes into existence. This field is called mechanistic interpretability and was able to deliver various milestones in the past years [list some of Antrhopic's work]. To enable similar studies in biological intelligence, we will need to construct "interrogateable" models of biological brains in-silico. This will allow scientists to explore the unifying patterns and differences in intelligence. Given that evolutionary processes had to evolve in a vastly more resource constrained environments than modern AI training runs, biological brains had to select for efficient designs - not only from an energy perspective, but also from a compression perspective, i.e. variety and complexity of tasks performed per network size. Comparative intelligence studies could port such designs to AI models following the pattern of "learning from nature" that finds applications across engineering [Data on nature inspired engineering]. Given the increasing presence of IOT devices the market of extremely efficient AI embedded in those will likely boost the already massive [IOT market data].

[Extrapolate to what could be intensified in the future - what is the concrete recommendation if any to the stakeholder + Comparative advantages versus other stakeholders]

Governments have provided in-depth reports with reflections on how to move forward. They saw big benefits in incentivising collaboration in a fairly segregated and highly interdisciplinary field. (HBP Final Report, 2024), the NIH's BRAIN 2.0 report refers multiple times to how public-private partnerships ought to be leveraged to help bridge the gap between academic innovation and commercialization. The continuation of funding via usual grant structure will continue the steady progress on advancements already made. A particular emphasis is to develop broadly accessible data repostiories, open infrastructure standards, etc (HBP Final Report, 2024)

In addition to these insights we recommend:

- **In-depth analysis of the potential economic impacts:** a) Commission a study on the effects investments in neuroscience have had already. b) Make an analysis that helps politicians and decision makers grasp the potential of investing in brain emulation technologies. Such an analysis should include assessments of the direct benefits (jobs created by investments) as well as the long lasting ones on the economy. A "The first Human in Cyberspace" plan could be a compelling mission to rally substantial resources behind and put a society at the frontier of a field that could have similar scale to Internet or AI.
- **Intensify Public-private partnerships:** While partnerships do already exist they could be further intensive similar to approaches followed by Defense Innovation Unit of the US' DoD (ref) where start-ups are enabled to raise additional funds based on government milestone payments. For instance, parts of the data that the scientific community would leverage could be bought by governments from start-ups.
- **Exploring centralized vehicles for advancing the field:** Given the necessary infrastructure, it is likely that a consortium based approach will limit advances substantially. Given the scale of mammalian brains, dedicated facilities as sketched out in the previous sections will be necessary.
- **Nudge highly skilled individuals to immigrate:** Get them to be part of accelerated visa approval programs. Explicitly invite them. Also provide them with excellent facilities to work from at universities.

Industry

In the previous chapters we gestured at the economic potential brain emulation technology can unleash for societies and that generously funded centralized efforts will likely have an advantage orchestrating enormous infrastructure, technology stack, and personnel count required for mammalian brain emulation efforts. In the best case companies are exactly that - a single organization focused on a shared mission.

While academia excels at fostering basic research in neuroscience, for-profit industry possesses distinct advantages in executing and scaling focused technological projects, most notably greater operational freedom and fewer bureaucratic constraints. Companies can quickly pivot on hiring, finances, and product strategies based on results. They can also simply engage in more and different types of funding, management structures, and procurement. Perhaps most importantly, industry's profit motive and market orientation provide natural mechanisms for efficiency and practical outcomes. Clear metrics for success and failure, coupled with market feedback, help ground large projects and force them to be efficient in a way that isn't always seen in government-funded brain initiatives.

A variety of industry stakeholders that exemplify the current involvement across the different brain emulation domains:

Neurodynamics: Within the last decade companies raised over 2B dollars, mostly in the context of brain computer interfaces and brain implants. Brain Initiatives T4 v3.xlsx
These include:

1. Neuralink has advanced high-density neural recording capabilities and surgical robotics
2. Kernel has developed non-invasive brain recording technologies
3. Synchron has pioneered minimally invasive neural interfaces
4. Paradromics and Blackrock Neurotech have developed high-channel-count recording systems

Connectomics Some technologies underpinning emulation that were developed in academia have been spun out into successful technology companies already. For example, Panluminate is commercializing light-based expansion microscopy developed at Yale. Zetta AI is commercializing connectome-tracing technology developed in the Seung Lab at Princeton.

Computational Neuroscience: Many other large tech companies are investing in neuromorphic computing, and could pivot once initial traction is gained in the field. These include Intel's neuromorphic computing initiatives (Loihi), IBM's TrueNorth architecture, and various AI chip companies developing neural accelerators. Besides, ongoing investments in better transistors, storage and communication bandwidth systems continue.

These are investments into subsections but not in the end-to-end brain emulation effort. While the advent of AI already introduces scalable intelligence, it leaves many motivations open for companies to develop intelligence that works and functions like humans - digital human intelligence, that are closely related to mission statement of existing successful companies. SpaceX wants to make humanity interstellar to maintain humanity—DHI could radically facilitate that by removing biological constraints of space travel. Longevity companies target aging and solve mortality, which—pending personal identity questions—would be de facto accomplished by personality-preserving brain emulation. Companies like Meta want to provide unprecedented experiences in virtual worlds—brain emulation companies could host such worlds and provide people with the respective infrastructure.

Beyond, investments in brain emulation will build a bridge between biology and an increasingly virtual economy. We can better embed the human perspective, the human experience, and our diverse cultures into this future if we meet the bar of reconstructing human intelligence as the ultimate measure of understanding it.

Venture capital and investors are no stranger to such visionary statements. They regularly overcome massive development costs—as long as there is a convincing case to be made for the risks taken providing high returns. Digital intelligence as a market is likely going to get saturated by (specialized) AI, since individual tasks can be most cost-efficiently solved with tailored AI models. But if brain emulation technology becomes so advanced that it allows creating personality-preserving in silico human representations, this would open up an unprecedented market not captured by any current technology.

Importantly, long-term investments are common and they created some of the most valuable companies on the planet:

OpenAI's journey represents a remarkable example of long-term scientific investment yielding transformative results. Founded in 2015 as a non-profit research laboratory, the company spent its early years conducting fundamental research across multiple AI domains while making significant theoretical contributions to the field. The company's commitment to iterative development and fundamental research, even without immediate commercial applications, culminated in the release of ChatGPT in late 2022 – nearly eight years after founding. For

OpenAI, the initial commitment in 2015 was $1 billion, primarily from early backers including Microsoft, Sam Altman, and others. Between 2015 and 2022 (pre-ChatGPT), the company raised approximately $11 billion in additional funding, with Microsoft being a major investor. Adjusting these investments for inflation, the total pre-profit investment would be approximately $13.5 billion in 2024 dollars.

SpaceX represents a compelling case. From its founding in 2002, it took nearly a decade before successfully launching and recovering its first rocket in 2012. During this period, the company faced multiple failed launches and near-bankruptcy. What made SpaceX's achievement remarkable was not just creating reusable rockets, but fundamentally transforming the economics of space travel. The company reduced launch costs by roughly 90% compared to traditional providers, enabling a new era of commercial space activity. For SpaceX, the initial investment period from 2002 to around 2012 (first successful recovery) required approximately $1 billion in direct investment. Elon Musk invested about $100 million of his own money in the early years (2002-2006), which would be equivalent to about $155 million in 2024 dollars. The total investment through 2012, adjusted for inflation, would be approximately $1.4 billion in 2024 dollars before the company began generating significant revenue from commercial launches.

In summary, it took 100s of millions of initial investments and eventually billions to get to the first product that then generated massive traction. Brain emulation pattern matches this sequence of events. Initial investments will be large, results will take years. Investors look far beyond the n-of-1 and ask what unit economics evolve once technical milestones are achieved. The price of the first brain emulation is less important (given some constraints) than how many customers exist that can pay for the 10th, 100th or millionth brain emulation.

The following unit-economics outline should be seen as a naive model that deserves a far more rigorous follow-up. It seems not inconceivable that the unit economics could fall from billions, to the order of magnitude of a yearly US household income in 2024 (census.gov, 2024). Readers are encouraged to use the calculator built based on the data collected for the report (see figure below) to model their own assumptions.

- **Neurodynamics:** As outlined in previous chapters the emulation of a brain of human scale depends on structure to function approaches. Accordingly invasive or non-invasive recordings are going to be disregarded for this exemplary analysis.
- **Connectomics:** Speculations about the initial costs of reconstructing a human brain span orders of magnitude and are highly dependent on the approach. Given current approaches, economies of scale and substantial advances in proofreading, the costs for brute-forcing the first connectome is - depending on the approach - in a left-tailed distribution between 1-100B dollars. The existence of such a reference connectome likely will enable rapid learning curves for other technologies and translate into quick declines in costs. Examples include the usage of dedicated high-throughput microscopes, leveraging substantially faster X-ray tomography, AI-driven compression and proofreading approaches, automation via robotics, and more. DNA sequencing costs fell by 7 orders of magnitude, from 8 billion dollars for the Human Genome Project (Tripp and Grueber, 2011), to $600 today (BIS Research, 2024). Given economic incentives, cost improvements by 4 to 6 orders of magnitude over two decades might be plausible and could drive down connectome cost to around $100,000.
- **Computational Neuroscience:** Estimates on exact compute requirements for different organisms are sparse. Assuming 1 exaflop in operations, 1 petabyte of storage, and 1 terabit of interconnect, systems like the NVIDIA DGX GH200, announced in 2023 and likely in the 10s of millions of dollars (Ref), are getting reasonably close across all dimensions at normal speeds. Accordingly, if a brain simulation was available in the next decade, the initial hardware costs will likely be in the low millions to 10s of millions. Given the lack of optimization for brain simulations, potential of neuromorphic compute and the general trend of falling computational costs (Hobbhahn, 2022) it seems not implausible that the eventual yearly hardware costs can fall to $10k or lower per person.

The following is a bottom up calculation of the total addressable market, where each phase is ~5y apart from each other.

Wealth globally (Visual Capitalist, 2022), with 3,194 Billionaires (Statista, 2022)

Net Worth	Number of Adults	Share of Adults	Total Wealth	Share of Global Wealth
More than $1 million	59.4M	1.1%	$208.3T	45.8%
$100,000 to $1 million	642.0M	12.0%	$178.9T	39.4%
$10,000 to $100,000	1.8B	34.4%	$61.9T	13.6%
Less than $10,000	2.8B	52.5%	$5.3T	1.2%

Total Addressable Market

Ultra High Net Worth Individuals

Global: ~3,194 billionaires
Plus ~34,000 individuals with >$100M

US: ~735 billionaires
Plus ~8,500 individuals with >$100M

Serviceable Available Market

UHNWI interested in technological immortality

Global: ~3,700 individuals
(~10% of UHNWI population)

US: ~925 individuals
(~10% of UHNWI population)

Pioneer Adopters

First 5 years of technology availability

Low Case (1%)
Global: ~37 individuals

High Case (3%)
Global: ~111 individuals

Low Case (1%)
US: ~9 individuals

High Case (3%)
US: ~28 individuals

Pioneer Phase Cost

Per individual brain emulation

Initial Cost (Year 1-2)
~$100M per person

Late Pioneer Cost (Year 4-5)
~$25M per person

Total Pioneer Phase Market Size

Global Low Case
~$925M
(37 × $25M)

Global High Case
~$2.78B
(111 × $25M)

US Low Case
~$225M
(9 × $25M)

US High Case
~$700M
(28 × $25M)

Total Addressable Market

High Net Worth Individuals (HNWI) Globally

Global: ~22.5M individuals
(Net worth >$1M)

US: ~7.5M individuals
(Net worth >$1M)

Serviceable Available Market

HNWI with interest in longevity/digital preservation

Global: ~4.5M individuals
(20% adoption rate)

US: ~1.5M individuals
(20% adoption rate)

Initial Market Penetration

Early adopters in first 5 years of commercialization

Low Case (2%)
Global: ~90K individuals

High Case (5%)
Global: ~225K individuals

Low Case (2%)
US: ~30K individuals

High Case (5%)
US: ~75K individuals

Estimated Service Cost

Per individual brain emulation

Initial Cost (Year 1-2)
~$2.5M per person

Mature Cost (Year 5+)
~$500K per person

Total Market Size (Year 5)

Global Low Case
~$45B
(90K × $500K)

Global High Case
~$112.5B
(225K × $500K)

US Low Case
~$15B
(30K × $500K)

US High Case
~$37.5B
(75K × $500K)

Total Addressable Market

Middle and Upper-Middle Class Households

Global: ~400M households
(Household income >$75K/year)

US: ~80M households
(Household income >$75K/year)

Serviceable Available Market

Households with sufficient savings & interest

Global: ~80M households
(20% of TAM with means/interest)

US: ~16M households
(20% of TAM with means/interest)

Initial Mass Market Adoption

First 5 years of mass market availability

Low Case (5%)
Global: ~4M households

High Case (15%)
Global: ~12M households

Low Case (5%)
US: ~800K households

High Case (15%)
US: ~2.4M households

Mass Market Phase Cost

Per individual brain emulation

Initial Mass Market Cost
~$250K per person

Mature Mass Market Cost
~$100K per person

Mass Market Total Size (Yearly)

Global Low Case
~$400B
(4M × $100K)

Global High Case
~$1.2T
(12M × $100K)

US Low Case
~$80B
(800K × $100K)

US High Case
~$240B
(2.4M × $100K)

Link to our Brain Emulation Calculator Mockup: The goal is to enable people to leverage all the data we have collected in this report and project out what the approximate costs would look like.

Neurodynamic Data

Data collection

Would be cool if the images are animated, i.e. the brain percentage is equivalent to the image, Hz maps to the max speed of the organism and then the selected Hz if shown in front of that.

Pull data:
- organism data for number of neurons & firing rate
- best tech depending on brain region and its today's characteristics: Percentage brain, recording speed, given behavior

Calculate
- Take the tech progress rates to determine when you will be able to start doing these experiments.
- From there onwards, over the overall time horizon given, calculate the number of Devices, Organisms and $

Depending on the organism some options will not be selectable, e.g. brain area for C. elegans, or > 1% human brain single Neuron recording.

Experiment 1

Some figure comparing EEG, fMRI and Single Neuron

Percentage brain

— 1 % +

Recording resolution

- (•) Brain area
- ( ) 1000s of Neurons
- ( ) Single Neurons

Recording Speed (Hz)

— 1 +

Behavior during recording

Fixated

Total Hours

1

[ ] (Optional) Neuromodulatory Effects

Some explanation of what this is.

+ Add Experiment

With more than 4 decades of basic technologies for brain emulation having passed and creation of technologies like expansion microscopy, neuronal labeling techniques, and large-scale functional recording, the baton may soon be passed from academia to industry. At this point

the first end-to-end brain emulation companies try to capture the first-mover advantage with intellectual property development and talent acquisition and retention.

The most notable company directly pursuing whole brain emulation is Eon Systems. Their approach combines high-resolution expansion microscopy imaging for connectome mapping with extensive functional imaging data to develop dynamic models. Aiming at mouse emulation, Eon represents one of the most ambitious commercial efforts in the space.

The main problem industry faces in contributing is lack of financially valuable intermediate products on the road to whole brain emulation. While industry has many advantages, it also has constraints that academia and others do not. In particular, the necessity of profit means that it is a poor fit for basic research that historically gave rise to important new emulation technologies like expansion microscopy, neuron barcoding, and connectome mapping techniques. The regulatory uncertainty and possible public backlash against the commercially valuable final product of digital humans could also slow investment . If either becomes a north star project that proves you can make money with brain emulation, massive investments are likely to follow.

Recommendations

- **Detailed report on brain emulation unit economics** over timeand the potential market sizes.
- **Determine addressable markets:** Market research on interest in brain emulation services across different demographics. What are the sizes of potential addressable markets?
- **Determine funding requirements:** Determine the requirements for a minimum viable signal that could convince investors to deploy on the order of billions of dollars. This likely will include convincing demonstration of personality-preserving characteristics in animal emulations with a strong evidence base for extrapolation to human scale.
- **Bootstrap start-ups from academia:** Most important for industry players is finding product-market fit in the value chain. One proven technique is to begin with academic technology that is used by others, and provide a scalable commercial solution. Spinning startups out of labs should be highly recommended. While academics don't always

know the corporate playbook, partnering with for-profit professionals can help a new initiative to deploy the best tactics from both academia and industry.

- **Provide the field with crucial technical estimates:** Many of the most advanced systems and the upper bounds of their capabilities are not public and thereby distort the sense of what is possible. For example: What is the maximum imaging rate of an electron microscope with dedicated development for connectomics and what do economies of scale look like? Having such estimates available will inform many other stakeholders about the general feasibility of brain emulation as a whole and accordingly enable the field.
- **OpenAI for Brain Emulation:** Convince investors of the upsides of financing long-term (5-10y) R&D in the 100s of millions, likely billions of dollars need to be deployed over the span of 5-10y.

Philanthropy

Throughout history, philanthropy has played a pivotal role in advancing transformative scientific endeavors, particularly those requiring sustained investment before delivering concrete returns or commercially viable applications emerge. Unlike government funding, which often shifts with political priorities and operates on rigid grant cycles, philanthropic capital can maintain unwavering support across decades. This patience proves particularly valuable for brain emulation, where development timelines may stretch 5-20 years or more. Similarly, while industry must prioritize near-term commercial applications, philanthropy can focus on developing fundamental tools and infrastructure that benefit the entire field. Philanthropy can serve as a neutral convener, bringing together diverse stakeholders and aligning their efforts. Similarly, the ethical implications and societal impact of brain emulation technology require careful consideration and public engagement – areas where philanthropy's mission-driven focus provides valuable perspective.

This is possible because endowments are solely committed to their founding mission:

- Simons Foundation: “providing sustained support to researchers working to unravel the mysteries of the universe. Through our work we make space for scientific discovery.” ref, 1990s

- Allen institute "transform human health through science for generations to come." ref, initiated in 2003
- HHMI: "to advance knowledge in the basic sciences for the benefit of humanity." ref 1953
- Wellcome Trust "support science to solve the urgent health challenges facing everyone." ref, 1936
- Tianqiao and Chrissy Chen Institute: ""drives fundamental brain research through in three key areas – brain discovery, brain treatment, and brain development." ref, founded 2016
- Chan Zuckerberg Foundation "build a more inclusive, just, and healthy future for everyone." ref, 2015
- Amaranth "engage skilled researchers and support ambitious ideas in the longevity and neuroscience fields"
- Foresight "advancing frontier biotech, neurotech, nanotech, and AI for the benefit of life." ref 1986
- Fieldcrest
- Brain preservation foundation "promote validated scientific research and technical services development in the field of whole brain preservation for long-term static storage." (ref)

(Current State of Philanthropic Investment) The current landscape of philanthropic investment in brain emulation-related work spans from large institutional funders to smaller foundations and individual philanthropists. The Allen Institute for Brain Science, established in 2003 with an initial $100 million commitment from Paul Allen, exemplifies institutional philanthropy's potential impact. The Institute has transformed neuroscience through its standardized, large-scale approach to brain mapping and commitment to open data sharing. Their work on mouse brain atlases and standardized experimental protocols has become foundational infrastructure for the field. ref

Several other major philanthropic institutions have made substantial contributions to neuroscience and brain-related research. The Howard Hughes Medical Institute (HHMI), established in 1953 (ref), has been a cornerstone of basic science funding through its investigator program and research facilities like Janelia, where much of the work on the fruitfly

happened. The Wellcome Trust (ref), dating back to 1936, has consistently supported neuroscience research and recently produced influential analyses of brain mapping feasibility and requirements. The Simons Foundation, founded in the 1990s, has advanced our understanding of neural circuits through targeted research programs and collaborative networks (ref). More recent entrants include the Tianqiao and Chrissy Chen Institute, established in 2016 with a 1 billion dollar endowment to drive fundamental brain research across discovery, treatment, and development (ref), and the Chan Zuckerberg Initiative, which launched in 2015 with a broader mission that includes substantial neuroscience funding (ref). The Amaranth Foundation has focused specifically on supporting ambitious neuroscience and longevity research projects (ref). Together, these organizations represent billions in philanthropic investment, though only a fraction directly supports brain emulation-related work. Their diverse approaches – from HHMI's emphasis on individual investigators to the Wellcome Trust's focus on large-scale collaborative projects – demonstrate different models for advancing complex scientific endeavors.

Smaller foundations and individual philanthropists have also made crucial contributions, often by identifying and supporting specific technological innovations. The Brain Preservation Foundation, despite its modest budget, has advanced key preservation techniques through targeted grants and prize competitions. These focused interventions demonstrate how even relatively small philanthropic investments can catalyze progress in critical areas.

A new model of philanthropic research organization has emerged through focused research organizations (FROs) like E11 Bio. These entities combine the mission-driven focus of philanthropy with the operational efficiency of industry. E11 Bio, for instance, is advancing molecular connectomics technology that could dramatically reduce the cost and complexity of brain mapping. This hybrid approach – neither purely academic nor commercial – demonstrates philanthropy's ability to pioneer novel organizational structures.

Technology Development

For instance, current efforts to develop protein-based barcoding systems for tracing neural connections require sustained investment in basic molecular tools before practical applications emerge. While individual labs might advance pieces of such technology, philanthropy can fund integrated development programs that combine molecular engineering, imaging technology,

and computational analysis. The Allen Institute's recent success with Multicolor Random Access Reconstruction demonstrates how such coordinated technology development can transform the field.

Importantly, philanthropy can ensure these technological advances benefit the entire research community by supporting open-source development and standardization efforts. The impact of the Allen Institute's standardized experimental protocols and data sharing platforms illustrates how shared infrastructure can accelerate progress across the field. Similar opportunities exist for developing standardized tissue preparation methods, imaging protocols, and computational tools for brain mapping and simulation.

Organizational Innovation

Beyond funding specific technologies, philanthropy can pioneer novel research structures that overcome traditional institutional limitations. The focused research organization (FRO) model, exemplified by E11 Bio, demonstrates how combining academic freedom with industrial operational efficiency can accelerate progress on defined technical challenges. This approach proves particularly valuable for brain emulation, where success requires coordinating diverse expertise and maintaining focus over extended periods.

Training programs represent another key leverage point. The field requires researchers who can work across traditional disciplinary boundaries – combining expertise in neuroscience, physics, computer science, and engineering. Philanthropy can support novel training programs that cultivate this multidisciplinary expertise. The HHMI's Janelia Research Campus provides an instructive model, creating an environment where early-career scientists develop broad technical capabilities while working on focused research challenges.

Field Building

Perhaps philanthropy's most distinctive contribution lies in building the field's long-term foundations. This includes supporting development of ethical frameworks and public engagement strategies – crucial work that often falls between the cracks of traditional funding sources.

Two major philanthropic initiatives demonstrate different but complementary approaches to advancing complex scientific fields. The Allen Institute for Brain Science, established in 2003 with an initial $100 million commitment, transformed neuroscience by introducing industrial-scale approaches to basic research. Their standardized protocols and comprehensive datasets, particularly the Allen Mouse Brain Atlas, have become fundamental resources cited in over 8,000 scientific papers. The Institute's open science approach and emphasis on creating research infrastructure that benefits the entire field led to breakthrough tools like the Allen Cell Types Database, which provides detailed molecular and physiological data for thousands of neurons. The Howard Hughes Medical Institute's investigator program, launched in 1984, took a different approach by providing long-term, flexible funding to exceptional scientists. This model has proven remarkably successful - HHMI investigators have won 32 Nobel Prizes since the program's inception, including Jennifer Doudna's 2020 award for CRISPR gene editing and Richard Axel's 2004 prize for work on olfactory receptors. Their seven-year renewable grants, often exceeding $1 million annually per investigator, enable scientists to pursue ambitious research directions that might seem too risky for traditional funding sources. Together, these examples demonstrate how philanthropy can advance scientific fields through both institutional infrastructure development and support for individual scientific excellence. The Allen Institute's success in standardizing protocols and sharing data, combined with HHMI's track record of enabling breakthrough discoveries, provides a powerful model for supporting brain emulation technology development.

Recommendations:

- **Data Collection on the Brain emulation field:** Fund a OurWorldInData / EpochAI for Brain emulation that focuses on aggregating high quality data across the field such as status of costs and speed of neuron reconstruction, simulation metrics, functional recording capabilities in different organisms.
- **Host challenges** that are easy to contribute to. This could include
    - providing compute for improving proofreading efforts, compression
    - - Lossless Data compression challenge: Datasets are massive and even with good internet connections not easily accessible. Improving lossless compression of different Neuro files would be not only huge for decentralized innovation, but

also necessary for larger scale efforts such as human connectome acquisition. Here too, differen modalities could be intermediate milestones
    - - Multimodal brain models that can flawlessly convert between different data modalities such as fMRI, calcium imaging, EEG, etc. E.g. using existing aligned datasets and contrastive learning. This could be a follow-up to the previous two, as it benefits from having the data in the first place...
    - - AI proofreading challenge where you want to go up to superhuman. We are already 70x better than what the Wellcome Trust report says, but getting it down further is the goal.
    - - Embodied fruitfly that can interact with its digital environment using the Flywire connectome. Intermediate milestones could be single behaviors and eventually all of the top 10 behaviors of the fly. Same could be tried with c.elegans as well.
    -
- **Explore regulation of digital minds:** Think ahead and explore spaces that are currently not top of mind.
- **Build a job board and call for proposal database:** Aggregation of all open calls / jobs for people to contribute to.
- **CommonCrawl for Neuroscience:** Leverage existing repositories to build "Common Crawl for Neuroscience" - a dataset that is cleaned (ref), standardized and can be used by everyone without to advance Neuroscientific research. It would also push the ecosystem to adopt more standards. Milestones could be to start with certain modalities and eventually move to a “all neuroscientific data publicly available"
- **Initiatives on reporting standards** This report made it evident that there is very inconsistent reporting of metrics across publications. Developing standards (e.g. these 15 variables for experiments) and lobying journals / communities to enforce checklists similar to those in clinical trials (EQUATOR, 2024) will improve comparability of studies substantially.
- **Track how many people are working on the topics:** Unclear how big the community really is.
- **Brain Emulation advocates**
    - Brain emulation advocates / stewards - Meta Community aka “Program Management Department” that continuously pulls from all stakeholders

- A lot of the vectors are not aligned and people are doing something that might be helpful for brain emulation, but not optimized for brain emulation solely → Backchain from emulation
    - Different people are interested in different kinds of brain emulation and have different motivations engaging in the topic which causes vectors to point into different directions. Finding a common denominator could be extremely powerful
- Help to identify the bottlenecks in the field and explain them clearly
- Interdisciplinary group of people
- Create clarity and enable different groups
- Fill the gaps that no individual party can take ownership of
- Headhunt excellent talent for key roles in the ecosystem?
- Help Develop more detailed research proposals based on the above deliverables with SME from Academia
- Things that come to mind
    - Create this report and further iterations to help everyone with excellent resources
    - Drive conversations around the ethical and societal implications
    - Help to develop intentional terminology
    - Help to develop comprehensive roadmaps together with SMEs
    - Foster interdisciplinary approaches
    - Provide clarity on funding opportunities
    - Brain emulation in data hub
    - ...

Gap Analysis

Before discussing what is necessary to advance towards human scale brain emulations, we will discuss proposals for concrete experimental milestones and budget estimates for different organisms. Describe in detail some potential experiments, their costs and best-case timelines.

Then we'll outline what a brain facility would likely look like that could meet the scale of the human brain.

Organisms

In the following chapters we highlight the core motivations and limitations of pursuing further advancement of brain simulations for the respective model organism. We then present a gap analysis.

All efforts should chain backwards from the specific needs of computational neuroscientists working on the various simulation approaches, for examples data gaps they are facing with regards to characteristics of specific ion channels or certain thresholds of hours of standardized recording across specific behaviors. Accordingly, for this section we start wit computational neuroscience, follow with neurodynamics and connectomics, and conclude with overarching remarks for the organism. Proposals for improvement are based on the discussion / outlook sections of the papers referenced in this report. Derived projects are examples of potential deliverables that could advance the field. Numbers that underlie budget estimates can be found in the Appendix.

C. elegans

Gap Analysis by Domain

Computational Neuroscience

Current computational approaches include basic biophysical models of individual neurons, preliminary whole-brain simulations through the OpenWorm project, machine learning approaches for neuronal activity prediction, and initial frameworks for multi-scale modeling. However, these models inadequately integrate molecular signaling and

- Limited Experimental Electrophysiological Data: Large portions of the worm's nervous system have only partial biophysical parameterization, i.e. missing parameters for ion-channels that are used in simulation approaches. (Zhao et al., 2024; Sarma et al., 2018)
- Multi-lab Synergy instead of Heterogeneous and uncoordinated datasets: Data sourced for emulation attempts is scattered rather than systematically acquired in standardized formats. This slows the overall process substantially. Although many groups research C. elegans, no labs has all the expertise and technology (e.g., advanced calcium or voltage imaging, computational physics, etc.) required to build a fully integrated simulation. (Larson, 2021)
- Integrate neuropeptide data: Currently neuropeptides are not accounted for in any simulation approaches.
- Long-Horizon Predictive Capabilities: Current models struggle with extended predictions.
- Very limited behavior repertoire and sensory feedback loops:

Examples of potential projects that could advance the field substantially:

- What we need to emulate C. elegans: Concrete list of variables that are needed listed by computational neuroscientists that can be created by students / PhDs / research groups. $0.2M within 0.5 years
- Follow-up project to fill in the gaps in electrophysiology: 2.5 years, $2-3M
- Porposal for "C. elegans Emulation Institute"
- More elaborate scaling law results - TBD
- Integration Architecture for integrating neuropeptides
- Expand across all behaviors for the worm, 1y, $1M

Neurodynamics

The current state of neurodynamics in C. elegans includes whole-brain calcium imaging in freely moving animals, single-neuron manipulation via optogenetics, and the development of nuclear calcium indicator strains for long-term imaging. Basic voltage indicators have been demonstrated.

- Voltage Imaging Technology: Current rhodopsin-based voltage sensors are limited to small fields of view, limiting whole-brain voltage imaging capabilities. Additionally, voltage imaging can detect rapid events like action potentials, the signal-to-noise ratio remains a challenge, particularly in C. elegans neurons which often exhibit only small membrane potential changes (Hasehmi et al. 2019)
- Calcium Imaging: Nuclear-localized GCaMP, which enables brain-wide recordings, provides more restricted spatial and temporal resolution compared to other approaches. This affects our ability to capture fast neural dynamics and precise timing of neural events. (Atanas et al. 2023)
- Integration of Multiple Modalities: While some progress has been made in simultaneous calcium and voltage imaging (Tokunaga et al. 2024), current approaches require complex optical setups and careful correction of photobleaching effects. The ability to correlate different measures of neural activity (calcium, voltage, and neurotransmitter release) remains limited.
- Long-term Recording Capabilities: The development of nuclear calcium indicator strains has improved long-term imaging capabilities, but there remains a need for better tools to study neural dynamics across different timescales and behavioral states (Atanas et al. 2023).
- Circuit Manipulation Precision: There is a need for improved tools that allow simultaneous manipulation and recording from multiple identified neurons (Piatkevich 2023).
- Neuropeptide Signaling Resolution: While recent advances have provided insights into peptide-receptor interactions (Beets et al. 2023), the technology is still in its infancy and there is a need for tools to visualize neuropeptide signaling in vivo and understand its temporal dynamics in neural circuit function (Watteyne et al. 2024).

Examples of potential projects that could advance the field substantially:

- Perturbation-free experiments: By automating handling of individual C. elegans using microfluidics, using a spinning-disk confocal microscopy, labs would perform C.

elegans whole-brain imaging of ~200 freely behaving animals. Given the somewhat limited behavioral repertoire of worms, 200 hours of data could be sufficient, although 1,000 hours of data would be more advisable. Timeline: 2 years, $1-2M

- Optogenetic perturbation experiments: Activate single neurons, and measure the behavior and changes in all other neurons at 10 Hz using a lightsheet microscope, across roughly 2,000 animals. This way 1,000 - 2,000 hours of imaging data could be generated. This might generate up to a Petabyte of data though after processing, this should fall by close to two orders of magnitude. Timeline: 2 years, $1-2M

[Potentially: What is the fields capacity? How many labs seem to be working on this? Is this attainable?]

Connectomics

The connectomics field for C. elegans has produced multiple complete adult connectomes, developmental series data, initial molecular annotations, and synaptic strength estimates. However, several critical gaps remain.

- Relevance of synaptic variability: Researchers have observed that even for identically aged hermaphrodites, as many as forty to fifty percent of the synaptic connections can differ from worm to worm (Brittin et al., 2021; Cook et al., 2019). This means that two worms will share most of the same underlying "scaffolding" (i.e., which neurons physically touch), but their synaptic connectivity at those touches may be slightly different. The relevance for simulation approaches is unclear at this point.
- Gap Junctions: gap junctions are often incompletely captured. Improved methods are needed for consistent visualization and annotation of electrical synapses across samples. (Witvliet et al., 2021)
- Integration of Multiple Modalities: Current approaches typically analyze either connectivity or activity patterns separately. To bridge structure-function relationships integrated datasets that combine connectomic reconstruction with activity imaging and behavioral correlates are needed.
- We lack robust methods for studying dynamic connectivity changes: the molecular underpinnings that guide the selective strengthening and formation of certain synaptic contacts, alongside the relative stability of others, remain only partially understood

(Brittin et al., 2021; Cook et al., 2019). Finally, there is growing recognition that extrasynaptic signaling (including neuropeptides) and gap junctions can be just as critical for circuit function, yet these elements have often been incomplete in legacy datasets (Witvliet et al., 2021).

- Advance the scale of Expansion Microscopy annotation: [elaborate - TLDR: we currently can do X, eventually we likely want to do at least Y. In case someone has a good number + reasons to target that'd be great]

[Placeholder for anything that comes up:

- We struggled to find much about XYZ, accordingly studies about that are needed.
- Additionally, expert conversations pointed us to XYZ.
- Our bottom up calculations bring us to the conclusion that XYZ is needed.

]

Examples of potential projects that could advance the field substantially:

- The "definitive" gap junction resolution connectome, 2–2.5 years is anticipated, with a budget of $1–2M
- The 100 worm connectome project (study variability)
- Development of functional dynamics methods (TBD)

Integrated Program Proposal

In a 2021 Interview, the long-time lead Stephen Larson advocated that the C. elegans emulation ecosystem would highly benefit from an integrative approach, the "Allen Institute for C. elegans research", that brings the broad range of interdisciplinary experts together (Larson, 2021). In 2023, (Haspel et al., 2023) proposed systematically determining the input-output functions of every neuron through comprehensive perturbation experiments and "sample all combinations of presynaptic neurons" in conjunction with various other data collection efforts to fully reverse engineering the entire C. elegans nervous system and simulating its full range of behaviors. The project assumes it will need thousands of hours of experiments, but has not disclosed specific numbers or funding proposals publicly.

It is clear that all C. elegans emulation efforts will benefit substantially from neat coordination between neuronal recording and consequent reconstruction of the worms' connectome. An

upper bound of what could be achieved is a hours of neuronal recording per worm and subsequent connectome reconstructions in 100s if not 1,000s of individuals, testing differen resolutions and molecular annotation approaches.

It seems that a concentrated, sustainably funded effort could within 2-5 years provide conclusive answers to the many of the points raised above. The range of $5-50M, depending on scope and ambition and could mark a transition away from grants aimed at narrow research questions (e.g., genetics, single-neuron knockouts) towards a large-scale integrative project across subject matter experts. The concept of a focused research organization might be applicable here.

Drosophila

Gap Analysis by Domain

Computational Neuroscience

Current computational approaches include connectome-constrained whole-brain simulations using LIF neurons (Shiu et al., 2024), circuit-specific models of visual processing (Lappalainen et al., 2024), and embodied models for behavioral control (NeuroMechFly, Janelia fly). However, several critical gaps remain:

- Missing Molecular Foundation: While the FlyWire connectome provides complete structural connectivity, current models must bridge a significant structure-to-function gap through multiple assumptions, including neurotransmitter identities (Eckstein et al., 2024), synaptic strengths and more.
- "Integrated Neuromechanical Framework": Current tools typically separately simulate neural activity and biomechanics. Need either unified simulation platforms or standardized interfaces to integrate these components for whole-organism modeling.
- Validation Challenges: Despite demonstrations of specific behaviors like feeding and grooming (Shiu et al., 2024), there is no consensus on what constitutes success for a whole-brain Drosophila simulation. This gap between technical capability and evaluation frameworks risks limiting the field's ability to make systematic progress toward more accurate simulations.

Examples of potential projects that could advance the field substantially:

- Holistic “Simulate the fly” roadmap
- Something about out of distribution behavior testing frameworks ?
- More work similar to the one of Lappalainen, modeling specific circuits with tasks
- Expanding Shiu et al work to a) more behaviors b) more sophisticated simulation approaches
- Rambling: Some sort of robustness analysis that details how much of the brain’s function / structure can be changed and you still get the behavior you are expecting (narrowing down how large the error bar is we are operating under)
- OpenFly framework that aggregates all the computational neuroscience data and provides tools for everyone to use?
- "Comprehensive Validation Framework": Develop standardized benchmarks for evaluating whole-brain simulations, including quantitative metrics for behavioral accuracy, neural activity patterns, and response to perturbations. Establish an open platform for cross-lab validation. Timeline: 2-3 years, $2-4M.
- "Embodied Brain Simulation": Integrate connectome-constrained whole-brain models with detailed biomechanical simulations (like NeuroMechFly), enabling direct comparison between simulated and real fly behavior during complex tasks. Timeline: 2-3 years, $2-4M.

Connectomics

While the FlyWire project has delivered a complete adult brain connectome (Dorkenwald et al., 2024), several critical gaps remain:

- Complete CNS: As of now only parts of brain / ventral cord have been reconstructed from different organisms. A complete reconstruction of the central nervous system is needed.
- Molecular Limitations: The FlyWire connectome, derived from electron microscopy, provides only morphological and connectivity information, lacking crucial details about neurotransmitter identities, receptor distributions, and other molecular properties that influence circuit function.
- Molecular Annotation Gap: Despite promising technical advances - including re-PKA-ExM achieving EM-like resolution via >40× expansion (Tian et al., 2024) and Bitbow achieving over 32,000 barcodes (Li et al., 2021) - no group has yet attempted to

integrate these approaches towards creating a comprehensive ExM-derived molecularly annotated connectome. Beyond basic neurotransmitter identification, comprehensive mapping of receptor subtypes and their specific locations remains critical, as different receptor subtypes can produce distinct effects even with the same neurotransmitter.

- Inter-individual Variability: While there are indications of stereotypy in the fly brain's neural circuits, systematic understanding remains limited as the community has reconstructed little more than one "and a half" connectomes to date. And it does not yet account for differences in sex.

Examples of potential projects that could advance the field substantially:

- “Whole Fly CNS” - for both males and females reconstruct 4-5 connectomes of the whole CNS. Combine this with extensive calcium imaging prior to reconstruction. This would be roughly equivalent to $1mm^3$ which a single modern EM can scan within a year. Due to advances in proofreading tracing costs will likely be substantially lower. 2-3 years, 2-3M
- (brainstorming) “Whole Body EM” - just scan the whole organism and trace nerves with more detail across the organism. Probably 10M over 3-5y
- Something something “solve proofreading for the fly” with AI systems such that any new scanned connectome can be proofread asap.
- "Whole-Brain Combinatorial Barcoding": Develop genetic strategy combining orthogonal recombinases and multi-value cassettes to generate >200,000 unique molecular barcodes, enabling unambiguous labeling of all neurons in the Drosophila brain. Validate barcode diversity and readout compatibility with expansion microscopy. Timeline: 2-3 years, $2-4M.
- "Multiplexed Molecular Mapping Protocol": Develop protocol for iterative antibody labeling and imaging compatible with high-expansion (>40×) ExM in Drosophila brain tissue. Enable reliable detection of 20+ proteins through serial rounds of staining.
- "Synaptic-Scale X-ray Microscopy": Demonstrate 15nm (to enable tracing of the finest neurites) resolution imaging of Drosophila brain tissue using X-ray ptychography and other phase contrast methods, with focus on resolving individual synaptic structures and validating against electron microscopy ground truth. Establish feasibility for future whole-brain imaging applications. Timeline: 1-2 years, $2-3M.

Neurodynamics

Although significant progress has been made in functional imaging in behaving flies, significant limitations remain when aiming to capture whole-brain activity at high spatiotemporal resolution:

- Tracheal System Constraints: The presence of air-filled tracheae creates significant challenges for whole-brain imaging, as light scattering and aberration limit imaging depth and resolution. The fly's tracheal system creates significant challenges for whole-brain functional recordings, requiring trade-offs between imaging depth, resolution, and coverage that are more severe than in transparent organisms like C. elegans or larval zebrafish. As a result, current models generally rely on either partial or whole-brain structural data, supplemented by limited functional recordings from specific brain regions.
- Imaging Trade-offs: Current approaches navigate these challenges through different compromises. Light field microscopy achieves fast (~200Hz) whole-brain imaging but at sub-neuropil resolution. Two-photon microscopy provides cellular resolution but usually requires head fixation and has a limited field of view. Three-photon microscopy shows promise for deeper imaging through trachea-filled tissue but has not yet achieved whole-brain coverage.
- Behavioral Recording Constraints: Current imaging approaches restrict the behavioral repertoire that can be studied. This limitation is significant for Drosophila, where many of the behaviors that make it an attractive model organism - including courtship, for example - are challenging to study under current imaging conditions.
- "Local Field Potential Characterization": While LFP oscillations have been measured and correlated with sensory input, their computational role remains poorly understood, particularly in structures like the lamin
- Get more clarity on neuromodulation in Drosophila
- Advance the frontier of maximum recording time in Drosophila: Currently up to ~10h are possible. Given that the flies can get up to 75d old, potentially up to an order of magnitude more / fly might be possible.

Examples of potential projects that could advance the field substantially:

- "Deep-Brain Three-Photon Performance": Systematically characterize resolution, signal-to-noise ratio, and photodamage limits of three-photon microscopy in deepest

regions of the Drosophila brain. Establish performance benchmarks for imaging through trachea-filled tissue at cellular resolution. Timeline: 1-2 years, $1-3M.

- "Whole-Brain Voltage Dynamics": Optimize next-generation voltage indicators and three-photon microscopy for simultaneous recording from >10,000 neurons across multiple Drosophila brain regions at millisecond resolution. Timeline: 2-3 years, $2-4M.
- Develop some genetic variants that are easier to image? Do the transparency trick as with mice
- Develop some way of imaging flies in complex behaviors, e.g. super lightweight versions of the helmets mice have for continuous brain recording.
- Develop sophisticated repeated imaging abilities for fruitflies that allow for 25h+ of recording per individual

Integrated program proposal

Like larval zebrafish, Drosophila represents a unique convergence of experimental tractability and biological sophistication that makes it an attractive target for integrated brain simulation efforts. The need for such integrated approaches has been previously recognized - in a comprehensive 2021 analysis, Scheffer and Meinertzhagen outlined 15 key areas requiring coordinated investigation beyond connectomics, spanning biochemistry, cell physiology, and whole-animal concerns (Scheffer and Meinertzhagen, 2021). Both organisms sit at the frontier of current technological capabilities, and both are starting to leverage larger and larger consortia. The arguments for an expanded integrated program are thus equally compelling: success in either organism could validate whole-brain emulation approaches broadly and attract substantial investment toward more complex organisms.

The primary technical challenge facing Drosophila-based approaches stems from its semi-transparency. Unlike the larval zebrafish's essentially transparent brain, the presence of air-filled tracheae in Drosophila creates significant challenges for whole-brain imaging at cellular resolution. This limitation is particularly relevant given that one of the key challenges on the path to human brain emulation involves learning to bridge the structure-to-function gap. Additionally, as a vertebrate, the larval zebrafish might provide more directly relevant insights for mammalian connectomics and neural computation.

However, Drosophila has a broader research community, with more sophisticated genetic tools including combinatorial barcoding libraries already being close to achieving whole-brain coverage (Bitbow's 32,000+ barcodes) and expansion microscopy protocols achieving electron microscopy-like spatial resolution (>40× expansion with Re-PKA-ExM). Most significantly, Drosophila represents a stable adult stage organism rather than a rapidly developing larva, and thus could potentially support neural recording in the same individuals over longer time horizons. This eliminates many of the constraints that characterize larval zebrafish work, where the brief developmental window limits experiment duration and complicates data integration across modalities. Furthermore, adult Drosophila exhibits a substantially richer behavioral repertoire including sophisticated social behaviors. This behavioral sophistication could provide more stringent validation criteria for whole-brain Drosophila simulations, including better tests of out-of-domain generalization.

We estimate that a comprehensive program targeting whole-brain emulation in Drosophila would require $30-45M over 3-4 years. This includes: development of improved three-photon microscopy systems for whole-brain imaging through trachea-filled tissue ($8-10M), expansion microscopy infrastructure for molecular annotation ($3-4M), personnel costs including 20-25 PhD-level scientists and 12-15 technicians across experimental and computational domains ($14-20M), computing infrastructure and data storage ($3-6M), and wet lab consumables including transgenic lines, reagents, and animals ($2-5M).

Zebrafish

Gap Analysis by Domain

Computational Neuroscience

Current computational approaches include circuit-specific models based on partial reconstructions – e.g., the oculomotor circuit – (Vishwanathan et al., 2024), data-driven models leveraging whole-brain calcium imaging (Anonymous et al., 2024), and embodied models integrating sensory processing with biomechanics (Liu et al., 2024). However, several key limitations remain:

- Missing Structural Foundation: The absence of a fully proofread connectome forces whole-brain models to rely primarily on functional data or partial circuit reconstructions. Even when structural data exists, it lacks crucial molecular information about neurotransmitter types and receptor distributions. Without these biological constraints, models must make significant assumptions about basic circuit properties, limiting their biological accuracy.
- Behavioral Recording Constraints: Most high-resolution neural recordings rely on head-fixed preparations that restrict natural behavior. While recent advances enable tail movement in virtual environments or use mirror-based tracking systems for freely moving fish, these methods compromise either spatial or temporal resolution. Current setups particularly struggle with complex three-dimensional behaviors like hunting, limiting the ecological validity of collected neural data.
- Limited Functional Data: Current datasets are predominantly short-duration recordings from head-fixed larvae. The temporal resolution of calcium imaging (1-4 Hz) is roughly two orders of magnitude too slow to capture the fastest neural events, while whole-brain voltage imaging remains technically challenging and thus far comes with very short session duration. Cross-individual datasets are sparse, complicating efforts to test model generalization and establish circuit stereotypy.
- Immature Model Development: Existing whole-brain models are few and currently lack detailed biophysical implementation. The field has not yet established what level of biological detail is necessary for effective simulation, partly because there's no consensus on how to evaluate these emulations. No standardized benchmarks or success metrics exist, making it difficult to compare approaches or determine when a model is "good enough." The lack of systematic perturbation data (e.g., comprehensive optogenetic studies) further complicates model validation.
- Integration Challenges: Despite recognition of the issue from the research community, no effort has yet succeeded in capturing both in-vivo neural activity and detailed structural data from the same organism. This integration challenge is made particularly acute in the larval zebrafish due to its rapid development, as structural and functional properties can change significantly even within days.

Examples of potential projects that could advance the field substantially:

- Port OpenWorm to the Zebrafish Larvae (3D environments, databases with receptors, etc) as “OpenZebraFish”. Collaborate with OpenWorm on shared infrastructure and derive concrete asks for the data acquisition community based on a data gap analysis. Build it in such a way that it could eventually become a strong foundation for OpenHuman. Probably 2-3M over 1-2y
- "Whole-Brain Model Benchmark Consortium": Develop standardized benchmark tasks and metrics for evaluating zebrafish larvae simulations, including behavioral reproduction and neural dynamics matching. Timeline: 1-2 years, $0.3-5M.
- Structure to function Extrapolation validation: Once sophisticated data-sets are available evaluate whether structure to function extrapolation is possible by holding out different amounts of functional data and comparing model performances. Iterate further with molecularly annotated connectomes.
- "Connectome-Constrained Biophysical Simulator": Build pilot-level compartmental models for using the current version of the larval zebrafish whole-brain connectome. Timeline: 1 year, $0.2M.
- "Embodied ML Models of Zebrafish Behavior": Develop physics-based differentiable simulation environments and train biophysical models supporting auto differentiation to replicate specific behaviors (hunting, escape responses) using zebrafish behavioral data. Timeline: 2-3 years, $1.5-2.5M.
- Neural activity prediction scaling law study. TBD.

Connectomics

While whole-brain EM imaging at synaptic resolution has been achieved (Svara et al., 2022), several critical gaps remain:

- Reconstruction of the full CNS: Currently only the brain and not the spine is scanned.
- Reconstruction Bottlenecks: Although automated segmentation and synaptic detection of the Svara et al. dataset are complete, comprehensive proofreading remains ongoing. Parallel EM-based efforts face similar bottlenecks in reconstruction completion. The scale of manual intervention required for accurate proofreading continues to delay the availability of a fully reconstructed connectome. A fully reconstructed connectome could also significantly reduce the required perturbation studies by providing structural priors.

- Molecular Information Limitations: EM datasets provide only morphological and connectivity information, lacking crucial details about neurotransmitter identities and receptor distributions. While expansion microscopy (ExM) could enable both structural mapping and molecular annotation, no comprehensive whole-brain ExM effort has been completed in zebrafish. Viral barcoding approaches, potentially usable for molecular annotation in other model organisms, remain challenging in zebrafish due to insufficient time for protein expression during the brief larval stage.
- Developmental & Inter-individual Variability: The rapid development of larval zebrafish brains (typically studied at 5-7 days post-fertilization) creates significant registration challenges, as small age differences can lead to substantial structural changes. This complicates efforts to create a "standard" reference connectome and raises questions about circuit stereotypy across individuals. The lack of a standardized reference atlas further impedes cross-study alignment and comparison.
- Morphodynamic datasets
- Neuromodulation studies

Examples of potential projects that could advance the field substantially:

- "Zebrafish Connectome Proofreading Sprint": Accelerate proofreading of existing EM datasets through multi-lab collaboration or commercial partnerships, with regular partial releases. Timeline: 1-2 years, $2-5M.
- "Transgenic Barcoding Feasibility Study": Develop rapid expression strategies for molecular barcoding compatible with larval stage timing. Timeline: 1-2 years, $1-2M.
- "Whole-Brain ExM with Molecular Profiling": Create comprehensive expansion microscopy dataset with molecular annotations at synaptic resolution. Timeline: 24-36 months, $5-10M.
- 10x the number of connectomes, including brain and spine, to gather better understanding of different stages and interindividual differences. 15M over 2-5y,

Neurodynamics

While both calcium and voltage imaging have seen remarkable progress in larval zebrafish, with the transparency of the brain enabling whole-brain imaging approaches, several critical challenges persist:

- Spacetime Bandwidth Constraints: Current imaging techniques face fundamental trade-offs between spatial coverage and temporal resolution. One-photon light-sheet microscopy can image nearly all ~100,000 neurons but only at 1-4 Hz, while faster techniques like SCAPE microscopy (up to ~25 Hz) or voltage imaging (millisecond-scale) achieve higher temporal resolution at the cost of observing less than 10% of the brain. No current method achieves both whole-brain coverage and spike-timing resolution simultaneously.
- Behavioral Recording Constraints: Most high-resolution neural recordings rely on head-fixed preparations that restrict natural behavior. While recent advances enable tail movement in virtual environments or use mirror-based tracking systems for freely moving fish, these methods compromise either spatial or temporal resolution. Current setups particularly struggle with complex three-dimensional behaviors like hunting, limiting the ecological validity of collected neural data.
- Temporal and Developmental Limitations: The narrow developmental window (typically 5-7 days post-fertilization) constrains recording duration and consistency. Substantial physiological changes can occur within days or even hours, complicating long-term studies of neural dynamics and plasticity. This rapid development also challenges cross-individual comparisons, as slight variations in age or conditions can significantly alter neural and behavioral phenotypes.
- Perturbation Coverage: Systematic perturbation of all ~100,000 neurons remains intractable within the brief larval stage.

Examples of potential projects that could advance the field substantially:

- "Extended ZAPBench 2.0": Expand current whole-brain calcium imaging efforts to include recordings from more individuals and simultaneous high-resolution behavioral tracking (tail kinematics, eye movements) in head-fixed preparations. Develop standardized protocols for multi-lab data collection and sharing. Timeline: 2 years, $0.5-3M.
- “Whole-Brain Calcium Imaging Scale-up”: Get to higher rates for whole brain imaging in freely behaving individuals. 1-3 years, $1-5M
- "Whole-Brain Voltage Imaging Scale-Up": Advance current voltage imaging coverage from ~33% to >70% of all neurons while maintaining temporal resolution (>200 Hz),

through improved GEVI brightness/targeting and optimized microscopy. Timeline: 1-3 years, $1-4M.

- "3D Unrestricted Swimming Microscopy": Develop imaging systems specifically addressing the vertical movement limitation in current setups, enabling natural hunting behaviors where fish strike from below. Timeline: 3-4 years, $3-5M.
- "Comprehensive Perturbation Atlas": Systematic optogenetic perturbations with whole-brain activity recording. Map circuit-wide effects of activating/inhibiting defined neuron populations during specific behaviors. Timeline: 2-3 years, $2-3M.

Integrated program proposal

Unlike machine learning, where scaling laws help predict performance improvements from larger models & datasets, we lack clear metrics for how much structural annotation and functional data is needed for accurate simulation. This uncertainty extends to the level of molecular detail required, as we don't yet know whether this information is necessary for meaningful whole-brain simulation.

The larval zebrafish represents a unique convergence of experimental tractability and biological sophistication that makes it an unique target for integrated brain emulation efforts.

With reasonable effort truly accurate data across all dimensions of a vertebrate brain could be collected: Single neuron recording at peak firing rates, molecularly annotated connectomes, etc. This represents a best case scenario that can inform among other things upper bounds on emulation fidelities and how structural and molecular properties determine neural function. Both are invaluable for emulating larger brains, including the human brain, where whole-brain recording at single-neuron resolution is physically impossible and structure to function extrapolation will be required. A working prototype of a tiny, fully emulated vertebrate brain, could attract substantial investment from both venture capital and traditional funding sources, fundamentally transforming the funding landscape for brain emulation research and accelerating progress toward more complex organisms.

Given its potential, but also substantial technical development needs, the foremost step the community has to take is the development of a robust plan - an integrated zebrafish larvae

emulation roadmap. The scale of such an effort is likely less than one order of magnitude above the previously discussed C.elegans proposals but still suitable for the concept of mid-sized organizations such as FROs or even startups that want to take this as a validation step towards economically valuable vertebrate models. It's no surprise that the idea of an integrated Focused Research Organization dedicated to larval zebrafish has been floated within the research community.

We estimate that a comprehensive program along these lines would require $25-40M over 3-4 years. This includes: specialized microscopy systems for whole-brain voltage imaging ($6-8M), expansion microscopy infrastructure ($3-4M), personnel costs including 15-20 PhD-level scientists and 10-12 technicians across experimental and computational domains ($12-18M), computing infrastructure and data storage ($3-6M), and wet lab consumables including transgenic lines, reagents, and animals ($1-4M).

Mouse

Gap Analysis by Domain

Computational Neuroscience

Current computational approaches involve for the most part biophysically detailed models of specific circuits (cortical microcircuits, hippocampal networks), particularly those developed by the Blue Brain Project and Allen Institute. However, several critical gaps remain:

- **Models remain circuit- or region-specific**. Scaling simulations to the whole mouse brain poses substantial computational challenges. Further the field is bottlenecked by the absence of a whole-brain connectome able to constrain model architecture and parameters (Igarashi, 2024).
- **Structure-to-Function Translation Challenge**. The mouse represents the first organism where inferring function from structure becomes essential for brain simulation. Even with a complete connectome, translating structural connectivity into functional circuit dynamics poses fundamental challenges that will require the development of novel approaches (Abbott et al., 2020).

- **Limited Functional Data for Refinement and Validation.** Achieving the scale of functional data needed for comprehensive model refinement and validation remains a significant challenge, as current recording approaches capture only a fraction of the brain's neurons and attempts to increase scale face inherent trade-offs with signal quality and noise levels (Rupprecht, 2021).
- **Lack of Standardized Benchmarks.** While theoretical evaluation frameworks like the embodied Turing test have been proposed (Zador et al., 2023), practical implementation remains challenging. Even if whole-brain mouse simulations were achieved, without clear quantitative benchmarks, comparing results across different modeling approaches would remain difficult, creating a barrier for systematic progress.

Examples of potential projects that could advance the field substantially:

- **Virtual Reality Benchmarking Suite**: Develop standardized VR environments to test mouse neuromechanical models (e.g., DeepMind's biomechanical simulators) in naturalistic tasks like foraging, social interaction, and predator evasion.
- **Whole-Brain Simulation Performance Atlas**: Systematically analyze computational costs of simulating mouse-scale networks (70M neurons) across frameworks (Jaxley, BrainPy, NeuronGPU) at varying biophysical resolutions (LIF vs. HH models, different models of synaptic transmission etc).
- **Neuromechanical Integration Standards**: Develop open APIs and data formats to unify neural simulators (Jaxley, BrainPy) with biomechanical engines (MuJoCo, PyBullet). Enable bidirectional sensory-motor integration for embodied tasks like locomotion.

Connectomics

Recent advances in imaging technologies show promise for mouse connectomics: X-ray holographic nano-tomography (XNH) achieves synaptic resolution (Bosch et al., 2023), expansion microscopy (ExM) enables multiplexed staining of up to 20 proteins in expanded tissue (Tian et al., 2024), and national initiatives like BRAIN CONNECTS aim to map ~10 mm^3 of hippocampal tissue. However, fundamental bottlenecks persist in scaling these methods to the entire mouse brain (~500 mm^3).

- **EM-pipelines remain bottlenecked by proofreading requirements**. Electron microscopy (EM), the most mature imaging approach, faces prohibitive costs due to manual proofreading, which accounts for >95% of total project costs (Wellcome Trust Report, 2023). Even with AI-assisted segmentation, proofreading a whole mouse connectome would require prohibitive amounts of human labor. Alternative approaches like E11 Bio's PRISM (genetic barcoding + ExM) aim to bypass this bottleneck but remain unproven at scale.
- **Molecularly Annotated Connectomes**. EM provides only structural ("naked") connectomes, lacking synaptic-level molecular information. While ExM protocols now resolve ~20 proteins in expanded tissue (Tian et al., 2024), scaling this to whole-brain volumes requires orders-of-magnitude improvements in staining throughput, antibody compatibility, and automated analysis.
- **Inter-Individual Variability Unquantified.** Synaptic wiring varies across mice due to experience-dependent plasticity. Current efforts focus on single specimens, but modeling learning/memory requires mapping multiple connectomes—a task currently cost-prohibitive even for small volumes (Abbott et al., 2022).
- **Exascale Data Demands.** A whole-brain EM dataset (~1 exabyte) strains storage and analysis pipelines. Molecular annotation (e.g., protein labels) could expand this by 10–100×. While tools like EM-compressor reduce raw data needs by 128× (Li et al., 2024), they do not address the computational challenges of querying or analyzing petascale connectomes.
- **Alternatives to EM remain underdeveloped**. X-ray ptychography has demonstrated synaptic resolution (Bosch et al., 2023) but currently lacks throughput for whole-brain imaging. ExM has demonstrated molecular profiling but struggles with isotropic expansion and tissue distortion at scale. Correlative workflows (e.g., XRM-to-EM) remain experimental and labor-intensive (Jefferis et al., 2023).

Examples of potential projects that could advance the field substantially:

- **"Synaptic Receptor Necessity Study"**: Perform paired in-vivo electrophysiology and post-mortem expansion microscopy (ExM) to map synaptic receptor distributions (AMPA, NMDA, GABA, etc.) in the same neurons. Determine the minimal set of molecular markers required to predict synaptic properties. Publish open datasets linking receptor density, synapse size, and functional measurements.

- **AI Proofreading Scaling Laws**: Quantify how automated proofreading accuracy (c.f.., RoboEM) scales with training data volume and model size. Identify compute-optimal frontiers.
- **Ultra-Expansion Protocol Development**: Adapt re-PKA protocols to achieve 40–50× isotropic expansion in adult mouse brain tissue. Solve distortion challenges.

Neurodynamics

Recent advances include calcium imaging of ~1 million neurons in head-fixed mice via light beads microscopy (Manley et al., 2024), ~1,000 neurons in freely moving mice using miniaturized microscopes (Zong et al., 2022), and voltage imaging of ~300 neurons at 400 Hz (Bai et al., 2024). However, physical constraints and technical tradeoffs limit progress toward comprehensive whole-brain recordings.

- **Physical Limits of Whole-Brain Optical Imaging** Light scattering restricts cellular-resolution imaging to superficial layers (~1-1.5 mm depth), making subcortical and deep cortical circuits inaccessible (Marblestone et al., 2013). While novel approaches like tissue transparency agents (Ou et al., 2024) show promise, whole-brain imaging at cellular resolution remains physically infeasible due to unresolved scattering and absorption in deeper brain regions.
- **Harsh Tradeoffs Between Coverage, Resolution, and Noise**
  Current methods force extreme compromises. Head-fixed systems achieve high coverage (~1M neurons at 6-10 Hz) but restrict natural behavior. Systems compatible with freely moving behavior enable the study of naturalistic behavior but are only able to record about a thousand neurons. Voltage imaging resolves spikes at 400 Hz but is limited in coverage and recording duration (Bai et al., 2024). In general, Increasing coverage degrades signal-to-noise ratio (SNR) due to shot noise (Rupprecht, 2021), with setups compatible with free behavior suffering the steepest penalties.
- **Lack of Structural Integration.** Functional recordings are rarely paired with structural data from the same specimen. While integrative efforts like MICrONS combine in vivo calcium imaging with EM connectomics (MICrONS Consortium, 2024), these remain exceptions due to the prohibitive cost and time required for structural mapping. Inter-individual wiring variability further complicates cross-mouse registration, rendering most functional datasets "structurally ungrounded" and limiting their utility for validating computational models.

Examples of potential projects that could advance the field substantially:

- **Integrated Barcoding-Function Atlas**. Combine E11 Bio's PRISM with in-vivo calcium/voltage imaging to generate a multi-modal dataset linking neural activity patterns to post-mortem structural connectivity. Release open datasets pairing functional dynamics with synaptic connectivity maps.
- **Inverse Scattering Depth Benchmark**. Test the limits of deep-brain imaging by integrating Kang et al.'s inverse scattering algorithms with three-photon microscopy. Quantify maximum imaging depth in mouse brain. Compare performance to traditional two-photon microscopy.
- **Chronic Crystal Skull Endurance Study.** Systematically quantify the maximum viable recording duration using "crystal skull" glass windows in mice. Track signal quality over 6–12 months post-implantation.

Integrated program proposal

Unlike simpler organisms where comprehensive functional recordings remain feasible, the mouse brain represents a fundamental transition point where structure-to-function mapping becomes essential. With ~70 million neurons spread across complex three-dimensional structures, no current or foreseeable technology will enable simultaneous recording from all neurons at physiologically relevant timescales. This reality forces a pivot in approach: rather than pursuing exhaustive functional characterization, successful mouse brain emulation will require learning to predict neural dynamics from structural and molecular properties. The mouse thus serves as the critical test case for whether we can bridge the structure-to-function gap – a challenge that will only become more pressing as we move toward larger mammalian brains.

The scale of this challenge necessarily pushes beyond traditional academic approaches. Even focusing solely on structural mapping, a mouse connectome would require coordinated operation of 50+ electron microscopes running continuously for several years, generating exabytes of raw data requiring sophisticated storage and processing infrastructure. Molecular

annotation through expansion microscopy would demand similar scale-up in tissue processing and imaging capabilities. Functional validation would require multiple complementary approaches: large-scale electrophysiology arrays, advanced optical imaging setups, and extensive behavioral testing facilities. The computational infrastructure needed just to store and process this data would rival major scientific computing centers.

These requirements point toward the need for dedicated large-scale facilities – "brain fabs" that integrate industrial-scale tissue processing, automated imaging, high-performance computing, and extensive validation capabilities under one roof. Such facilities would need to operate more like semiconductor fabs than traditional neuroscience labs, with careful attention to throughput, quality control, and systematic data collection. While the initial investment would be substantial ($500M-1B for a prototype facility), this approach may be the only way to achieve the consistency and scale needed for meaningful progress toward mouse brain emulation.

This naturally raises strategic questions for the field. Given the resource requirements for even attempting mouse-scale emulation, should efforts first focus on more comprehensive characterization of simpler organisms like larval zebrafish or Drosophila? Or does the mouse represent such a critical test case for mammalian brain emulation that its challenges must be tackled directly? What seems clear is that pursuing mouse brain emulation requires a fundamental shift in approach – from distributed academic efforts toward coordinated, industrial-scale operations. Success would not only advance our understanding of mammalian brain function but also validate the infrastructure and approaches needed for larger-scale efforts. Failure would suggest the need to first master structure-to-function mapping in simpler systems where comprehensive functional validation remains feasible.

Human

Gap Analysis by Domain

Computational Neuroscience

Current computational approaches for the human brain are fundamentally limited by data availability. Most models are based either on low-resolution functional data (fMRI) or anatomical observations, requiring significant extrapolation from these sparse constraints. Several critical gaps remain:

- **Data Scarcity**: The most fundamental limitation is the lack of both functional and structural data to adequately constrain human brain models. While future technologies may eventually provide detailed structural data through connectomics, functional data at cellular resolution is likely to remain permanently out of reach due to physical and ethical constraints. This forces models to rely on massive extrapolations from animal studies or indirect measurements, severely limiting their biological validity.
- **Extreme Resource Demands**: Current attempts at human-scale simulations have yet to leverage state-of-the-art hardware architectures and simulation frameworks. However, even with optimal implementation, biophysically detailed models at true human scale (86B neurons with realistic synaptic connectivity) would require exascale computing systems for real-time simulation. The "memory wall" and interconnect bandwidth limitations pose particular challenges for efficiently simulating such massive, densely connected networks.
- **Model Validation Challenges**: Unlike model organisms where neural activity predictions can be directly validated against recordings, human brain simulations lack ground truth data for comparison. Behavioral validation alone cannot guarantee faithful replication of underlying neural mechanisms. We lack consensus on appropriate validation metrics, forcing reliance on indirect proxies like coarse-grained neuroimaging data or entirely novel validation frameworks that move beyond direct neural activity comparison.

Connectomics

Current connectomics approaches for the human brain remain limited to macro-scale diffusion MRI providing coarse structural information, and nano-scale EM studies of extremely small tissue samples. Several fundamental challenges remain:

- **Scale of the Challenge.** The human brain presents an unprecedented scaling challenge for connectomics, being more than three orders of magnitude larger than a mouse

brain. Achieving synaptic resolution in a volume of ~1,300 cm^3 (vs. ~0.5 cm^3 for the mouse) demands capacity and throughput far beyond the capabilities of current laboratories. Meeting this challenge will require near-industrial levels of coordination, equipment scale-up, and data infrastructure, elevating human connectomics from a traditional scientific project to a massive engineering and logistics undertaking.

- **Electron Microscopy (EM).** While electron microscopy remains the established gold standard for synapse-level connectomics, with an extensive track record in smaller organisms, scaling to the human brain faces prohibitive barriers. At current effective scan speeds, achieving a complete human EM dataset within a decade would require approximately 30,000 parallel electron microscopes (Jefferis et al., 2023), far exceeding global production capacities. Beyond microscope availability, sample preparation, automated image segmentation, and proofreading each introduce substantial computational and manual labor demands, though recent advances in machine learning have improved automation capabilities (Januszewski et al., 2020; Schmidt et al., 2021). Most critically, EM alone provides only a "naked" connectome—lacking direct information on neurotransmitter systems, receptor types, and other molecular properties essential for interpreting circuit function.
- **Expansion Microscopy (ExM).** Expansion microscopy has emerged as a promising optical alternative that can achieve nanometer-class resolution through physical tissue expansion. Recent demonstrations in mammalian systems (Rodrigues et al., 2024; E11 Bio, 2024) highlight ExM's potential advantages for large-volume imaging. ExM microscopes are significantly more cost-effective than EMs and rely on a broader, more mature supply chain. Methods in development may enable partial self-proofreading of neuronal tracts, and the technique is naturally compatible with molecular annotation, potentially overcoming EM's biggest limitation. However, ExM has never been attempted at anything close to the scale of a human brain. Tissue anisotropies, distortion, and batch-to-batch consistency remain unresolved issues, particularly at the extremely high expansion factors needed to achieve synapse-level resolution in large volumes.
- **X-ray Microscopy (XRM).** Fourth-generation synchrotron light sources are pushing X-ray microscopy toward an exciting frontier in large-volume,

high-resolution brain imaging. XRM promises high speed volumetric imaging of thick specimens, thanks to high-brightness synchrotron beams, and isn't constrained by the diffraction limit. However, XRM's practical viability for a full human connectome remains untested, with significant challenges in achieving dense reconstructions at synapse-level resolution. While X-ray fluorescence imaging may eventually enable multiplexed protein labeling, these protocols remain at an undeveloped stage.

- **Petascale to Zettascale Storage & Processing.** The data management challenges of human connectomics are staggering regardless of imaging approach. A complete human brain dataset would generate hundreds of exabytes to a zettabyte of raw data. Storing, transferring, and analyzing these volumes will require near-exascale computing clusters and extremely high-bandwidth infrastructure. Although emerging compression methods demonstrate promising data reduction capabilities (ref EMCompressor), such techniques must be integrated directly into imaging pipelines to be effective at full scale. Even then, the costs—in equipment, personnel, and operational complexity—pose enormous hurdles.
- **Structure-to-Function Dependency.** A human connectome's ultimate value for brain emulation depends critically on bridging the structure-to-function gap. A fully reconstructed wiring diagram, even with molecular annotations, is only the first step. Without validated computational tools to infer neural dynamics, emergent cognitive capabilities, and system-level behavior from structural data, the practical benefit for human brain simulation remains limited—especially given the near-absence of corresponding high-resolution functional data. Bridging this gap will require breakthroughs in both data integration and theoretical frameworks, highlighting the interplay between connectomics, neurodynamics, and computational modeling at human scale.

Neurodynamics

Current approaches to studying human brain dynamics face a fundamental dichotomy: non-invasive methods (fMRI, EEG, MEG) can achieve whole-brain coverage but lack single-neuron resolution, while invasive recordings can resolve individual neurons but are limited to a few thousand cells.

- **Physical Recording Limits.** Fundamental physical constraints create insurmountable barriers to achieving whole-brain, single-neuron resolution recording in humans (Marblestone et al., 2013). Issues of light scattering, signal attenuation, energy dissipation, and probe invasiveness make comprehensive neural activity mapping physically impossible with current or near-future technologies. These constraints aren't merely technical limitations awaiting an engineering solution, but represent basic physical bounds that cannot be circumvented through incremental improvements. Barring fundamental breakthroughs, single neuron resolution whole brain coverage in humans will remain out of reach.
- **Resolution vs Coverage Trade-off.** Non-invasive methods provide whole-brain coverage but at the cost of spatial and temporal resolution. fMRI measures slow hemodynamic responses averaged across hundreds of thousands of neurons, while EEG and MEG provide faster temporal sampling but even coarser spatial resolution. Conversely, invasive recordings can resolve individual neurons but are restricted to tiny fractions of brain tissue – at best capturing thousands of neurons compared to the brain's ~86 billion. This trade-off between resolution and coverage appears fundamental rather than technological, making comprehensive human brain recording permanently out of reach.

Integrated program proposal

Over the last four decades, the field of brain mapping has progressed from manual tracing of neurons in the C. elegans nervous system to the complete reconstruction of the Drosophila brain, increasingly comprehensive whole-brain imaging in larval zebrafish, and early efforts toward mouse connectomics. Yet the human brain sits in a league of its own—more than three orders of magnitude larger than the mouse brain, which itself dwarfs current large-scale mapping efforts by more than an order of magnitude. This extraordinary scale creates a fundamental divide in feasibility: while whole-brain recording at cellular resolution is achievable in zebrafish and likely feasible in Drosophila, and whole-cortex recording remains at least imaginable for the mouse, comprehensive neural recording in humans faces insurmountable physical barriers with current or near-future technologies, even setting aside regulatory and ethical challenges. This means that unlike more approachable organisms where

neural recordings can constrain brain simulations, human-scale models must rely primarily on structural datasets for model fitting because direct functional measurements at single-neuron resolution are physically unattainable for the foreseeable future. Bridging the "structure-to-function" gap thus becomes fundamental—without robust methods for predicting biophysical properties and circuit dynamics from structural and molecular data, even a perfectly reconstructed human connectome would remain insufficient for meaningful simulation.

Given current technology, human brain simulation efforts would require industrial-scale operations that dwarf any existing neuroscience facility. Imagine "brainfabs" the size of modern semiconductor manufacturing plants: thousands of electron or optical microscopes running in parallel, or entire synchrotron facilities with multiple beamlines dedicated to brain mapping. Such facilities would demand their own large-scale storage and computing infrastructure to process the multi-exabyte datasets generated daily. The resource requirements—likely running into billions if not tens of billions of dollars—push these efforts beyond the realm of academic institutions and into the domain of large-scale governmental or public-private initiatives.

Human brain emulation faces unique challenges that set it apart from other organisms. Yet the path forward, while daunting, is becoming clear. The physical impossibility of comprehensive neural recording means that success depends entirely on our ability to predict neural dynamics from structural data. This suggests a natural progression: first master structure-to-function translation in more approachable organisms where whole-brain recordings can validate our predictions. Only once we understand which molecular annotations are essential and how to reliably infer circuit dynamics from static measurements should we undertake the years-long process of imaging the human brain. While the scale of human brain mapping demands industrial approaches, the fundamental questions around structure-to-function must be settled before such massive investments can be justified.

[PREVIOUS VERSION START]

Across the board, the cost to map a single neuron has fallen drastically over the last 40 years. In the 1980s, it cost about $15 million, over a decade-long span, to map 312 neurons (Cost: 30 neurons per year per $1 million). For the same amount of money, we can map about ~50 000 Neurons today. Once we move the same relative distance up on the log-axis and hit 1.5M

neurons, we'll be able to reconstruct a human brain in less than 5 years for about $10B. Here the plan for how to get there. See figures.

(Neurodynamics) The initial recommendation is a total of 20,000 hours of fMRI across 50-200 subjects, alongside extensive auxiliary (e.g., fNIRs, EEG) data. Analysis of the Natural Scenes Dataset and Tang dataset indicates that a 10-fold increase in data size per subject would yield significant improvements in data analysis; the Natural Scenes Dataset contains 35 hours per subject, while Tang contains 20 hours per subject. The proposed approach increases data collection to 300 hours for both visual and auditory processing, with additional data from resting state, cognition, and memory tasks providing valuable insights. An adaptive, multistage data collection design would continuously optimize for brain state prediction performance. Beyond fMRI, the collection should include other brain state recordings like EEG and fNIRS, along with comprehensive sensory input, including interoceptive cues such as heart rate, respiratory rate, and proprioceptive cues. Motor output should be fully captured. While fMRI experiments routinely control and record audio-visual inputs with limited motor outputs, non-fMRI experiments like fNIRS allow subject movement. In these cases, mixed-reality headsets could capture visual inputs, while external cameras would record subject movements, complemented by movement sensors in the fNIRS "helmet." Analysis would also require critical information about attention and fatigue levels. Deep recordings of multimodal datasets, such as fMRI-fNIRS-EEG (Scarapicchia et al., 2017) may allow for efficient translation between modalities. Although there is no evidence to suggest that fMRI experiments are harmful, this volume of fMRI data has never before been collected, so the health of the participants must be carefully monitored. Assuming a cost of $1,000/hour, this volume of data collection would be a total cost of $20 million. Clinical studies in drug development and elsewhere frequently ask a lot of their subjects and can in the limit be substantially more expensive. [elaborate on complex clinical trials that have been performed in the past. Make the case that spending $50M on data acquisition is something done frequently]

(Connectomics) The Wellcome Trust released a report in 2023, estimating that it would cost between $7 and $21 billion (Wellcome Trust, 2023) to build a connectome for the mouse brain. The BRAIN CONNECTS project was recently funded by NIH and aims to scan 1/30th (~10-15 mm^3) of the mouse brain by 2028, while establishing the infrastructure for a

complete mouse brain connectome by 2033 ("BRAIN Initiative Connectivity", 2022). The project is funded with $150M over a five-year period.

Assuming a deadline of 10 years of imaging and using the 91-beam electron microscopes assumed in the Wellcome Trust Report, a human brain would take a hypothetical 30,000 electron microscopes at current speed running in parallel. Ignoring whether such a scale-up of the manufacturing base is feasible, with a cost of $1,000,000 a piece, this would cost $30B in just microscope costs — without operation costs, sample handling, or reconstruction. Our current microscopes are not fit for the vast scale of a human brain.

1.4e24 / 1e3 / 1.5e8 / 60 / 60 / 24 / 365.25 / 30,000 = ~10 years

Major EM manufacturers have not faced the demand necessary to produce such high-throughput microscopes. Responding to the demand seen by mouse connectome efforts, sufficiently large orders, or major projects towards human-scale connectomics, we would not be surprised of major innovation being latent in the electron microscope space. We estimate that improvements, most importantly in automated sample handling, reliability, and uptime could plausibly increase the speeds farther by 2-fold to 5-fold.

1.4e24 nm3 / (16*16*16) nm voxel size / 1e10 (ie 10 GHz) / 0.5 (50% uptime) / 60 / 60 / 24 / 365.25 / 10 years = ~200 EMs

I.e. 200 of such high-throughput 10-GHz mass-produced SEMs would be needed to image a human brain in roughly 10 years. With as little as a single major private or government order incentivizing manufacturers to move from a craftsmanship approach to mass-produced economies of scale, and charging closer to production cost of a microscope, experts (K. W.) estimate that cost a piece may remain in the million-dollar range, potentially as little as $1M. This gives 200 microscopes * $1M = $200M USD for the cost of microscopes to image a human brain in roughly 10 years.

Given the recent application to connectomics no calculations similar to the ones in the Wellcome trust report have been published yet. Performing similar protocols on a full mouse brain would increase the volume to that of ~four human brains.

[EM calculator](#).

[ExM calculator](#)

				Michael's estimate				
Paradigm			Dense-labelling	Dense-labelling	Dense-labelling	Barcoding	Barcoding	Dense-labelling
Brain specs	**Notes**	**Unit**	Zebrafish larva	Mouse	Mouse	Mouse	Human	Human
Brain size		mm3	0.1	400	500	500	1400000	1400000
Resolution required	Especially constrained by dense rec	mcm	0.015	0.032	0.015	0.05	0.05	0.015
Deadline	Within x days the imaging has to fin	days	120	120	365	365	1825	1825
Microscope specs								
Microscope name			ASI diSPIM	ASI diSPIM	ASI diSPIM	ASI diSPIM	ASI diSPIM	ASI diSPIM
Individual Microscope Cost	Assuming some economiees of sca	USD	$300,000	$240,000	$200,000	$200,000	$150,000	$130,000
Resolution lateral		mcm	0.4	0.38	0.38	0.38	0.38	0.38
Resolution axial	Axial resolution is assumed as the c	mcm	0.4	0.38	0.38	0.38	0.38	0.38
Imaging speed, cubic mm per hour		mm3/hour	5	9	5	5	6	6
Post-processing resolution boost	eg deconvolution, SIFT, ...	factor ≤ 1	1	1	0.9	0.9	0.9	0.8
Expansion factor required	Resolution / (Required resolution / r	factor	26.66666667	11.875	22.8	6.84	6.84	20.26666667
Brain size, post-expansion	Brain size * (Expansion factor ^3)	mm3	1896.296296	669824.2188	5926176	160006.752	448018905.6	11653999881
Multiplexing specs								
Total staining targets	not including every-round stains	number	30	4	6	30	30	6
Every-round fluorescent stains	eg PSD-95, synaptic markers, etc	number	2	1	1	1	2	2
Multiplexing per round	Assuming the microscope has thos	number	9	5	8	8	9	9
Multichannel slowdown	Within a round; slowdown factor of	factor	1.3	1	1.3	1.3	1.3	1.3
Rounds required	CEILING(Total stains / (multiplexing	number	5	1	1	5	5	1
Imaging days	(expanded volume / imaging speed	days	102.7160494	3101.03805	64200.24	8667.0324	20223075.6	105209721.2
Number of scopes needed	CEILING (imaging days / deadline)	number	1	26	176	24	11082	57650

We could establish a dedicated datacenter to handle storage and upload bandwidth similar to other large-scale scientific efforts such as CERN. In CERN's case, their facility has storage capacities exceeding >3 exabyte and throughput on the order of 100-200GB/s, which is more than enough to keep up with data generated from our microscope paths (20GB/s) [ref, source, (Jefferis et al., 2023)]. CERN's datacenter cost 83m CHF ($93m USD) to build, and on the order of $100-200m USD to run per year [source]. Assuming we build a data center with ~10 petabytes of storage, 100x smaller than CERN's, a rough cost estimate gets us to anywhere from $10-50 million for the initial build and compute costs [source]. Storage can be scaled up incrementally - see CERN's approach

Semiconductor chip manufacturing is a particularly good model, as it works on similar scales (nano-scale), deploys similar equipment (EMs), makes use of highly automated processes and meticulous quality control procedures. Our modern computer chips are manufactured in so-called "fabs" that have equipment of different abilities to produce chips of different capacity, commonly measured in nm, e.g. 180nm for less intensive computing and 3nm for cutting edge smartphone chips.

Current market estimates for electron microscopes are estimated around 3-5B dollars for 2024 (ref, ref). If we assume new system sales make up perhaps 40-50% of the market (a reasonable assumption for complex scientific equipment), and costs of 1-8M dollars, 500-2,000 EMs are sold each year. The market for electron microscopes is anticipated to rise along with the demand for materials science, semiconductors and life sciences. TSMC 10 to 20 chip facilities should deploy hundreds of EMs - and a single facility can have as many as 50(?). (ref.).

The semiconductor industry likely has access to better performing EMs, facilities are fully automated and reliably maintained, and work in substantially higher quantities. Neuroscience's semi-automated scanning causes effective scanning at 5% or less of the microscopes maximum capacity (Welcome Trust Report). Similar to other industries transitioning to more and more automated processes will come with substantial improvements.

Adopting scale and automation procedures from other industries will likely be akin to a seismic shift in the neuroscientific EM scanning landscape.

Processing this many biosamples sounds daunting. But again, there are industry examples that have already approached this problem. Modern pharmaceutical screening facilities demonstrate remarkable throughput capabilities. Leading acoustic liquid handlers can transfer hundreds of samples per second with nanoliter precision, while automated storage systems manage millions of samples at -80°C with retrieval rates of 500+ tubes per hour. These facilities can operate continuously with minimal human intervention, maintaining precise environmental conditions and chain of custody tracking. The integration of robotics, automated analysis, and AI-driven experimental design enables throughput that often exceeds traditional laboratory automation by orders of magnitude.

World's largest antibody manufacturing facilities (South Korea, Switzerland, China) have capacities of 300,000 - 350,000L that can produce tons of monoclonal antibodies annually in a single plant. (ref) Clinical Diagnostic Operators such as Roche Diagnostics process >15 million

immunoassays daily worldwide, with a single facility can handle >100,000 tests per day - fully automated systems with minimal human intervention (ref)

Inspired by this, we propose conceiving "brain fabs" that are an amalgamation of best practices in semiconductor industry, bio-pharamceutical scale tissue handling, large scale animal facilities and supercomputing. Such a facility would centralize the essential parts of data acquisition for brain emulation, enabling maximum cost competitiveness and efficiency.

Building on existing blueprints in other industries, such a facility could be designed in a modular manner, further preventing some of the worst issues modern day mega projects face. Solar or wind farm megaprojects continuously meet cost and time estimates and scientist who study why big projects fail attribute this amongst other things to their modularity. (Why big projects fail, 2023).

With extendability in mind the facility should be used to scan several more of the already reconstructed organisms (C. elegans, Fruitfly and Zebrafish) in conjunction with large scale electrical and chemical studies that have been discussed previously. We could scan 10s to 100s of individuals and create a much richer understanding about interindividual differences that will eventually be essential to correctly model brains. Once workflows are established, machinery can be upgraded and tissue samples can be replaced for those of mice.

Aiming for the moon can get you into stratosphere: The brain fab could build up to capacity that can do experiments and connectome reconstruction of 100 mice, again, collecting indispensable data to inform our understanding of mammalian brains across individuals and enable substantially more robust modelling.

In many ways computational neuroscience is strictly limited by the accessibility of easily accessible and usable datasets. A brain fab could provide these at unprecedented scales.

It sounds surprising, but scaling EM infrastructure to eventually image the human brain is in the reference class of a vastly simplified semiconductor facility dedicated to EMs, probably on the order of 100-200 at effective imaging rates of 10-15GHz, scanning fully automated brain

tissue samples for a time horizon and a price tag similar to the human genome project. (elaborate calculation available in the supplements).

So far neuroscience facilities are optimized for science, not for scale of data acquisition. Making a bet on scale is the necessary next steps to advance the field. It is simply implausible to generate consistent data or be cost competitive compared to a brain fab as an academic lab or even research campus such as Allen Institue or Janelia. Notably, such a facility, on the order of TSMC's Arizona plant, would likely create 10s of thousands of high-tech, construction and supplier jobs in potentially remote locations that could highly benefit.

The following is as precise a sketch of such a facility as we were able to create.

A figure of the Brain Fab - 3D visualization purpose built for facility for brain scanning in comparison to modern facilities like Tesla's Megafactory.

(Computational Neuroscience)

(potential failure modes)

Appendix

Funding opportunities summary

Funding Scale	Fieldbuilding	Data (Collection & Integration)	Technology (R&D)	Infrastructure	Finance & Contests
100k	- Roadmapping of various topics: technologies (Microscopes, Proofreading,...) Unkowns (Plasticity, Structure to function, etc), organisms (existing organisms, new model organisms, economic impact of past and future neuroscience - Multiday Workshops Series / Symposium similar to BRAIN Connects - Polishing of this current report with great publication / interactives towards a Brain emulation field data hub aggregator (Calculator, timelines, clean datasets, etc) - High-quality lecture series / online course	- Organism specific “Neurocrawl” of all publicly available datasets that can be easily used for computational neuroscience - Some high end multi modal datasets that include functional recording and structure in the same individual - Gaps in model organism data project - Project to leverage AI agents scanning the vast neuroscience space to generate new insights	- Small-scale tech pilots (e.g barcoding tests) in existing labs - Development of meaningful metrics and benchmarks for brain simulations / emulations	- Compute Credits to individual organizations / labs - Reporting Checklist for certain neuroscience publication to increase comparability between studies + implementation with Journals - Contributions to OpenWorm IT Infrastructure	- Challenges and prizes for e.g. “Best image compression approach” or “AI-based annotation” or "the most versatile Simulation" - Sponsorship of conference tracks focused on brain emulation (NeurIPS, SFN, ICLR, etc.)

1M	- Brainemulation fieldbuilding org that coordinates roadmaps, conferences etc. - Bootstrap an Epoch.ai / Our World in data for brain emulation platform - Governmental lobbying efforts - Technically rich Brain Emulation Roadmap - Planning of Large Scale Brain facilities	- Record hundreds of hours of fMRI, likely ten thousands of hours of non-invasive data for the respective model organisms - Expand Neurocrawl to data hub across multiple organisms with continuously updated pipelines and data cleaning - Launch a “Kaggle for Brain Emulation” (continuous public competition and training on neuro dataset challenges) with moderate platform budget. - C. elegans electrophysiology gap-filling project	- Targeted instrumentation prototypes (miniaturized 2P scopes, advanced viral barcoding). - AI-based segmentation or connectome-proofreading enhancements. - Run a pilot project to test advanced viral barcoding in C. elegans or larval zebrafish. - advancing 2P / 3P Imaging capabilities	- FRO for developing new organisms - Port OpenWorm infrastructure to various other organisms to simplify and standardize work for computational neuroscientists	- Larger prizes for similar domains - Seed funding in brain emulation orgs for initial strategy / org setup / hiring (for-profit / FRO) - Standard academic grants
10M	- Launch FROs, e.g. "Structure to function FRO” - Seed funding for University Institue for Brain emulation	- 10mm3 of mouse brain feasibility - Tighter integration: structure + function + behavior in single datasets - Interindividual comparison studies: 5-20 individuals recorded + scanned	- Industrial-scale prototypes: advanced 3-photon, next-gen ExM, or robust barcoding kits. - High-speed “X-ray tomography” or advanced “Voltage imaging” for small mammals. - Exploratory tech programs on current unkowns, eg. plasticity, identity defining aspects, glia, etc. - Demonstrate scaling laws in neuroscience based on newly generated massive datasets.	- Synchrotron beamline (initial build) - Mini-AWS for computational neuroscience: Provide access to compute and large scale datasets easily in one place for little to no costs.	- “X-prize” style challenge
100M	- Generously fund a Brain Emulation Org (similar to OpenAI model) - Launch Mouse FRO - DARPA-esk program on brain emulation	- “Mouse or marmoset” scanning facility Connectome	- Dedicated neuroscience microscopy chip development	- Full synchrotron or X-ray tomography center. - HPC expansions akin to “AWS for neuroscience.” - Centralized Brain Emulation Fab Prototype	- Venture fund on Brain emulation - “Advanced Market Commitment” guaranteeing $100M purchase if a certain resolution or throughput is met. - Major philanthropic endowment into the field
1B	- Possibly link with “BRAIN Initiative v3” or large philanthropic consortia, incl. Brain Emulation Institute - “OpenAI Series A” style mega-org with $1B–$2B for WBE over 5–10 yrs.	- Human connectome	- Next-gen “Neuromorphic” chip dev. - True “BrainFab prototype” with robotics, advanced scanning, etc.		
10B	- Human Genome / CERN-scale coalition: unify governments, philanthropy, industry for near-human mapping.		- Brain Fab for high-troughput brain emulation data acquisition		- Multi-billion “public–private” partnerships or major philanthropic campaigns akin to HGP or SpaceX.

Data

- Overview of Neuron Simulations - T3
- Funding - Brain initiatives, other projects
- Brain Initiatives T4 v3.xlsx
- Compute visualization
- Automatic Tracing Costs
- Neurodynamics recording papers

Funding opportunities 10x10

- Funding Opportunities Brain Emulation Landscape

Figures

- Datascience figures

Budget variables used in calculations

- Microscope: A single spinning disk confocal microscope would be around $500,000 - 750,000. Alternatively, rent a spinning disk for approximately $100/hr. Lightsheet microscope and microfluidics cost: $550,000
- PhD level scientists (each $150,000/year)
- Technicians (each $100,000)

Concrete proposal

Category	Prototype Facility	Full-Scale Facility
Total Footprint	25,000-30,000 m^2	200,000-250,000 m^2
Main Areas		

- Structural imaging facility	5,000 m^2	30,000 m^2
- Functional imaging facility	3,000 m^2	20,000 m^2
- Data center	4,000 m^2	30,000 m^2
- Animal facilities	3,000 m^2	40,000 m^2
- Sample processing	4,000 m^2	30,000 m^2
- Support infrastructure	4,000 m^2	50,000 m^2
- Administration/research	2,000 m^2	20,000 m^2
- Expansion space	5,000 m^2	30,000 m^2
Clean Room Areas		
- Class 100 (ISO 5)	2,000 m^2	20,000 m^2
- Class 1000 (ISO 6)	3,000 m^2	30,000 m^2
- Class 10,000 (ISO 7)	5,000 m^2	40,000 m^2
Power Requirements		
- Total peak capacity	40-50 MW	300-400 MW
- Data center	20 MW	150 MW
- Imaging systems	10 MW	100 MW
- Support systems	10 MW	100 MW
- Backup capacity	25 MW	200 MW
- Solar installation	10 MW peak	100 MW peak
- Battery storage	50 MWh	500 MWh
Animal Facility		

- Mice capacity	500 cages	2,500 cages
- Zebrafish tanks	200	1,000
- Drosophila vials	2,000	10,000
- C. elegans plates	10,000	50,000
- Surgical suites	2	10
- Behavioral testing rooms	4	20
Structural Imaging Equipment		
- Electron microscopes	20	200
- Expansion microscopy stations	2	20
- Sample prep robots	10	100
- Automated sectioning systems	5	50
- Quality control stations	3	30
Functional Imaging Equipment		
- Multi-photon microscopes	5	50
- Light-sheet microscopes	3	30
- Calcium imaging systems	10	100
- Voltage imaging setups	5	50
- fMRI systems (3T/7T)	1/0	5/2
- Functional ultrasound systems	5	50
- Behavioral recording setups	10	100
- Optogenetics stations	5	50

Computing Infrastructure		
- Compute capacity	500 Petaflops	5 Exaflops
- GPUs	1,000	10,000
- CPU cores	10,000	100,000
- Total storage	100 Petabytes	10 Exabytes
- Internal bandwidth	1 Tbps	10 Tbps
Sample Processing		
- Daily tissue processing	100 cm^3	1,000 cm^3
- Sample prep stations	10	100
- Automated staining systems	5	50
- Quality control stations	3	25
- Sample storage capacity	1,000 samples	10,000 samples
Functional Recording Capacity		
- Total recording hours	500 hours/day	5,000 hours/day
- Simultaneous recording rooms	4	40
- Multi-modal setups	2	20
- Real-time visualization stations	2	20
- Daily data generation	50 TB/day	500 TB/day
- Online processing capacity	100 PFLOPS	1 EFLOPS
- Active experiment storage	5 PB	50 PB
Staffing		

- Total staff	250-300	2,000-2,500
- Scientific staff	50	500
- Technical staff	100	1,000
- IT/Computing	50	300
- Maintenance	25	200
- Administrative	25	200
- Support services	50	300
Support Infrastructure		
- Liquid nitrogen production	200 L/day	2,000 L/day
- Ultra-pure water	10,000 L/day	100,000 L/day
- Compressed air	1,000 CFM	10,000 CFM
- Chemical storage	100 m^2	1,000 m^2
- Waste handling	500 m^2	5,000 m^2
- Loading docks	4 bays	20 bays
Annual Operating Costs		
- Power	$20-30M	$200-300M
- Staff	$30-40M	$300-400M
- Consumables	$20-30M	$200-300M
- Maintenance	$10-20M	$100-200M
- Total operating cost	~$100M	~$1B
Construction Costs		

- Building	$200-300M	$2-3B
- Equipment	$400-500M	$4-5B
- Infrastructure	$200-300M	$2-3B
- Total initial cost	$800M-1.1B	$8-11B

A concrete proposal for what the path might look like

For the sake of this exercise let's assume that scanning, processing and running a human brain is 100B dollars. This ought to be a proxy for all the costs involved: Employees, Training, Travel & Events, Consulting Services, Compute, Hardware, Licenses, Logistics, Materials & Consumables, Miscellaneous other things, Operational overhead, Quality management, Contingency Reserve (Budget allocated for identified risks within the project scope), Management Reserve (Funds set aside for unforeseen risks).

To break it down some further:

Category	Percentage	Amount	Notes
FTEs	35%	$35.0B	Largest expense - highly skilled workforce required
Compute	20%	$20.0B	Massive computational requirements for simulations
Hardware	12%	$12.0B	Specialized research equipment and infrastructure
Contingency Reserve	8%	$8.0B	For identified risks within scope
Management Reserve	5%	$5.0B	For unforeseen risks and challenges
Operational Overhead	5%	$5.0B	Facility operations and maintenance
Quality Management	3%	$3.0B	Validation and verification processes
Consulting Services	3%	$3.0B	External expertise and review
Materials & Consumables	2.5%	$2.5B	Research supplies and materials
Licenses	2%	$2.0B	Software and technology licenses
Training	2%	$2.0B	Staff development and training
Travel & Events	1%	$1.0B	Conferences and collaboration
Logistics	1%	$1.0B	Supply chain and distribution

Miscellaneous 0.5% $500M Other unforeseen expenses

Let's also assume that this will get 2% smaller every year, since technological advances make it cheaper to reach the same milestones (more microscopes / GPUs / etc for the same price).

We estimate the current cumulative budget on brain connectomics & emulation at $0.25B. It increases every year by 5%.

If you run several permutations of this, you get a distribution of outcomes. The important point here is that most of these are likely happening in this century.

Distribution of Intersection Years and Values

Let's illustrate this further and quantify what these numbers would mean in terms of number of people working on this, available resources using our previous constants (We start of with a assumed price for brain emulation of $100B pictured as a purple line. This bar gets 3% smaller every year, since technological advances make it cheaper to reach the same milestones.

Brain Emulation Project Resource Quantification (32 Years, $37.7B)

Category	Percentage	Amount	Resource Quantification
FTEs (Scientists, Engineers, Support Staff)	35%	$13.2B	~400-450 FTEs per year on average: - 150-175 Senior Scientists/Engineers ($300k/year) - 150-175 Junior Scientists/Engineers ($180k/year) - 100 Support Staff ($120k/year) Total personnel cost per year: ~$412M
Compute	20%	$7.5B	Equivalent to ~250-300 petaFLOP/s sustained over project: - 50,000-60,000 high-end GPUs - Power usage: 15-20 MW - $235M/year for hardware refresh + operations
Hardware	12%	$4.5B	Research Infrastructure: - 3-4 major research facilities ($1.5B) - Specialized imaging equipment ($1B) - Lab equipment & tools ($2B) - ~$141M/year for maintenance & upgrades
Contingency Reserve	8%	$3.0B	Risk mitigation fund: ~$94M/year
Management Reserve	5%	$1.9B	Unforeseen challenges fund: ~$59M/year
Operational Overhead	5%	$1.9B	Facility operations: ~$59M/year - Utilities - Maintenance - Security - Administrative support
Quality Management	3%	$1.1B	~$35M/year for validation processes and quality assurance teams
Consulting Services	3%	$1.1B	~$35M/year for external experts and reviewers
Materials & Consumables	2.5%	$942.5M	~$29M/year for research supplies and materials
Licenses	2%	$754M	~$24M/year for software and technology licenses
Training	2%	$754M	~$24M/year for staff development programs
Travel & Events	1%	$377M	~$12M/year for conferences and collaboration
Logistics	1%	$377M	~$12M/year for supply chain management
Miscellaneous	0.5%	$188.5M	~$6M/year for miscellaneous expenses
Total	**100%**	**$37.7B**	

To be continued...

– END –

Random Notes

Perhaps the most high-profile attempt at full-scale brain emulation is the Blue Brain Project (Markram, 2006). Spearheaded by Henry Markram at the École Polytechnique Fédérale de Lausanne (EPFL) in 2005, it promised to provide a detailed emulation of a cortical column within two years, and a full emulation of a rodent brain within a decade. The project ended unceremoniously in 2024, failing to deliver on its ambitious promises. To better understand this project, where it failed and where it succeeded, it is worth going back to examine the state of the art of 2005, two years before the first iPhone was released and only one year after the usage of GPUs for training began slowly thawing the prevailing AI winter (Oh and Jung, 2004).

The project developed from a collaboration between EPFL and IBM. IBM would provide the computational power in the form of their Blue Gene Supercomputer. Developed on the basis of the Deep Blue supercomputer, which had beat world chess champion Gary Kasparov just eight years earlier, Blue Gene delivered a whopping 3.56 petaFLOPs (Almasi et al., 2008) of peak performance (roughly 500-1,000x less than modern supercomputers / AI training datacenters). These would be used not only to simulate brain activity, but also to fit parameters of neuron models to existing data.

EPFL, on the other hand, would provide the neuroscientific expertise. As an accomplished electrophysiologist, Markram had made a name for himself by discovering calcium-transients in cells at voltages that did not give rise to action potentials (Markram & Sakmann, 1995) and their movement within the cell (Markram, Helm, Sakmann, 1995) as well as the importance of spike-timing on changes of connection strength (Markram et al., 1997). His group had helped

develop multi-neuron patch-clamp approaches, allowing the simultaneous recording of activity from hundreds of neurons (https://www.science.org/doi/10.1126/science.293.5531.868).

While the project never managed to emulate a full brain, it had partial success. The first large-scale simulation was published in 2015 (Markram et al., 2015), modelling a volume of 0.29 mm^3 of neocortex from a juvenile rat with over 31,000 neurons. It should be noted that at no time did the project have a complete connectome of this area. Instead, they developed methods to unite diverse datasets and reconstruct connectomics data from biological principles and known statistics. The proportion of different cell types, for example, was based on manual classification of 2,056 cells, while the number of cells was estimated by counting cell numbers in six individual rats. These cells were then placed at biologically plausible random locations within the volume and connections inferred based on biological rules (Reimann et al., 2015). Rather than modelling any individual rat, they therefore modelled a cortical column of one possible rat. The resulting simulation spontaneously exhibited activity similar to that measured in vivo, and could be validated by varying aspects of the simulation such as the concentration of extracellular Ca2+ and comparing the resulting changes with laboratory experiments.

How do you know whether a brain simulation is accurate? The Blue Brain Project validated its results by introducing simulated inputs and showing that activity statistics were close to those expected. Walker et al. (2019) pioneered a more direct approach: they fit deep neural networks to recreate the recorded activity of visual neurons in response to arbitrary visual stimuli. While the model itself was abstract and sacrificed biological plausibility, the predicted activity closely matched *in vivo* activity. They could then extract images that would theoretically produce the maximum excitation of certain model neurons, present these to mice and record neural responses, validating that the model accurately captures brain processing. Such early models showed promising performance on static visual images, but generalised poorly when applied to novel stimuli, and often did not have the data required to predict on dynamic visual stimuli (movies).

Image from [Walker et al., 2019]
The inception loop paradigm. By training a CNN to predict neural activity in response to natural images, Researchers can extract maximally exciting images (MEI) and validate model performance by measuring in vivo evoked activity.