Imaging whole-brain activity to understand behaviour

Albert Lin[1,2,3,4], *Daniel Witvliet*[1,2], *Luis Hernandez-Nunez*[2,5], *Scott W. Linderman*[6,7], *Aravinthan D. T. Samuel*[1,2] ✉ *and Vivek Venkatachalam*[2,8]

Abstract | Until now, most brain studies have focused on small numbers of neurons that interact in limited circuits, allowing analysis of individual computations or steps of neural processing. During behaviour, however, brain activity must integrate multiple circuits in different brain regions. Whole-brain recording with cellular resolution provides a new opportunity to dissect the neural basis of behaviour, but whole-brain activity is mutually contingent on behaviour itself, especially for natural behaviours such as navigation, mating or hunting, which require dynamic interaction between the animal, its environment and other animals. Many of the signalling and feedback pathways that animals use to guide behaviour only occur in freely moving animals. Recent technological advances have enabled whole-brain recording in small behaving animals including the nematode *Caenorhabditis elegans*, the fruit fly *Drosophila melanogaster* and the larval zebrafish *Danio rerio*. These whole-brain experiments capture neural activity with cellular resolution spanning sensory, decision-making and motor circuits, and thereby demand new theoretical approaches that integrate brain dynamics with behavioural dynamics. We review the experimental and theoretical methods used to understand animal behaviour and whole-brain activity, and the opportunities for physics to contribute to this emerging field of systems neuroscience.

[1]*Department of Physics, Harvard University, Cambridge, MA, USA.*

[2]*Center for Brain Science, Harvard University, Cambridge, MA, USA.*

[3]*Center for the Physics of Biological Function, Princeton University, Princeton, NJ, USA.*

[4]*Princeton Neuroscience Institute, Princeton University, Princeton, NJ, USA.*

[5]*Department of Molecular and Cellular Biology, Harvard University, Cambridge, MA, USA.*

[6]*Department of Statistics, Stanford University, Stanford, CA, USA.*

[7]*Wu Tsai Neurosciences Institute, Stanford University, Stanford, CA, USA.*

[8]*Department of Physics, Northeastern University, Boston, MA, USA.*

✉*e-mail: samuel@physics.harvard.edu*

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Animals possess repertoires of natural behaviours that allow them to navigate, interact with the environment and interact with other animals. Examples include searching for mates, hunting prey or escaping from predators. These behaviours require animals to simultaneously process many different sensory experiences, make different types of decisions on multiple timescales, and continuously monitor and modify their own movements and behavioural performance. Natural behaviours are not easily reduced to one-to-one mappings from sensory stimulus to motor output, as can be done for feedforward reflexes. Instead, natural behaviours engage many types of neural computation at the same time — multisensory processing, memory storage and recall, decision-making, motor production, feedback and control mechanisms — in ways that cannot be compartmentalized. These computations are often carried out by many brain areas acting together, communicating via system-wide networks of synaptic connectivity and non-synaptic modulation.

To understand the relationship between whole-brain activity and behaviour, ideally one would access the entire brain during behaviour with minimal artificial constraints. Although doing so is not generally possible, a few model organisms are suited to recording whole-brain activity in intact animals during natural behaviours. Small animals with transparent bodies and brains, such as nematodes, larval *Drosophila* and larval zebrafish, are natural candidates. The heads of non-transparent animals, including adult *Drosophila* and larger vertebrates, must be surgically opened to view the brain, or have microscopes inserted into the brain. In this Review, we focus on the nematode *Caenorhabditis elegans*, the fruit fly *Drosophila melanogaster* and the larval zebrafish *Danio rerio* (TABLE 1).

Other small animals, such as the hydra, are also being developed as models for whole-brain and whole-circuit approaches to behaviour[1]. In addition, there has been much recent work in rodents, for which large numbers of neurons can be recorded in rich behavioural contexts[2–5]. Studies in these animals allow circuit-level dissections of behaviour[6,7]. However, it is not yet possible to record from whole mammalian brains with full cellular resolution. The development of neuropixel electrodes has increased the throughput of electrophysiological brain recordings in mammals, but without the full field of view and resolution of microscopy systems[8]. Functional magnetic resonance imaging (fMRI) can visualize whole-brain activity based on changes in blood flow to different brain regions, but has low spatial resolution compared with optical methods and is not a direct measurement of neuron activity[9,10]. We thus limit

Key points

- Advances in optical microscopy allow brain-wide imaging with cellular resolution throughout the sensory, decision-making and motor circuits of behaving animals.
- A complete understanding brain-wide dynamics requires requires the context provided by behavioural dynamics: ongoing behaviour emerges from brain activity, and brain activity itself is contingent on past behaviours and experiences.
- Brain activity is organized by structural, functional and physiological mechanisms. The wiring diagram of the brain (the connectome) represents pathways of synaptic information flow. The molecular properties of synapses and cells determine the neuronal responses to sensory and synaptic inputs. Non-synaptic mechanisms organize brain-wide activities corresponding to different behavioural states.
- Small animals like nematodes, insects and larval fish are tractable models for comprehensively exploring and modelling the mechanisms of brain-wide activity and behaviour.
- Modelling brain-wide activity is a multiscale problem from synapses to cells to circuits, across brain areas and across behaviours.
- Both top-to-bottom modelling — posing a theory of neural computation and modelling biological mechanisms that might carry it out — and bottom-to-top modelling — looking for structure in high-dimensional activity patterns that might explain correlated behavioural patterns — are important strategies for building towards an understanding of brain-wide dynamics and animal behaviour.

our discussions to imaging approaches to whole-brain activity in behaving animals.

The capacity to comprehensively record the brains of worms, flies and fish during behaviour arose with recent developments in microscopy (FIG. 1). Fast, high-throughput microscopes combine rapid volumetric imaging with 3D tracking of brain-wide dynamics. Many of these imaging systems are also capable of simultaneously monitoring the behavioural dynamics in unrestrained, semi-restrained or virtual reality experimental setups. Advances in imaging technology and data analysis will continue to expand the range of possible experiments, allowing the acquisition of complete brain recordings during more types of behaviour[11].

Systems neuroscience in worms, flies and fish is now generating rich data sets of brain-wide activity that span multiple sensory inputs, distributed circuits and different behaviours. Understanding these data sets requires innovation in theory and computation. Questions that arise include: whether fully mapping the detailed patterns of co-variation between sensory inputs, brain activity and motor responses is enough to understand the brain; whether there are principles of integrated brain function that impose low-dimensional structure on the correlations between sensation, cognition and action; and how anatomical wiring imposes constraints that can be used to better understand brain and behavioural dynamics.

In this Review, we describe the technological advances that have enabled rich recordings of whole-brain activity and behaviour. We discuss recent experiments in model organisms that have captured behaviourally relevant brain-wide activity, as well as computational and theoretical approaches that attempt to link brain activity to behaviour. At each stage, we highlight ways in which physicists have contributed to this field and the opportunities for future work.

Methods for whole-brain imaging

Experimental methods. On timescales shorter than a second and spatial scales longer than hundreds of micrometres, diffusion is too slow to synchronize cellular or system-wide activity. To coordinate the activity of sensory and motor systems over long distances, neurons rapidly propagate electrical signals along fibres throughout the nervous system. Electrical signalling is coupled to changes in the intracellular concentrations of multiple ions, including calcium. These changes are typically followed by the activation of intracellular signalling pathways and eventually cell-to-cell communication by short-range synaptic transmission and long-range neuromodulation[12]. Therefore, measuring activity at the whole-brain level requires microscopic probes that can globally detect changes in electric fields, intracellular ion concentration or neurotransmitter release. Genetically encoded sensors derived from fluorescent proteins have been developed for all of these aspects of neuronal activity[13–16]. One of the most successful approaches has been to use microscopy to capture activity-dependent fluorescence from proteins expressed in neurons of transgenic animals. The studies we review here primarily use the GCaMP family of indicators, derived from green fluorescent protein (GFP). Many years of engineering and ever wider use of the GCaMP family of indicators have led to improved stability, sensitivity and signalling properties in each new version, making GCaMP ideal for stable, long-term imaging of large populations of neurons in many genetically accessible animals[2].

After choosing a fluorescent sensor, microscopes are needed that can both resolve single cells throughout the brain and also sample at informative timescales of behavioural and neuronal activity, from milliseconds to minutes or longer. The most common approach to imaging many cells with single-cell resolution using fluorescence is to confine the excitation light to a portion of the imaging volume, selectively capture in-focus light from that portion, and then serially scan the brain volume. This approach, known as point scanning, underlies confocal, two-photon, structured illumination, and light-sheet microscopy[17–21].

Point scanning has advantages in optical resolution, but is typically too slow to image many cells throughout a large brain volume on subsecond timescales. Whereas conventional two-photon and confocal approaches use point scanning to image a brain volume, other approaches accelerate the scanning. Confocal microscopy can be accelerated by simultaneously scanning many points in a focal plane using a 2D array of pinholes (spinning-disk confocal microscopy). Two-photon laser scanning microscopy (2PLSM) allows deeper imaging into larger brains and can be accelerated by adaptive, closed-loop scanning to improve image acquisition speed for behaving animals[22,23].

Living biological samples are generally more susceptible to photodamage than inanimate samples when subjected to laser light. Light-sheet microscopy confines light to an imaging plane without allowing propagation into parallel planes, allowing optical sectioning with minimal photodamage. Many light-sheet microscopes use separate objectives for delivering excitation light

and recording fluorescence; doing so imposes physical constraints on the animal being recorded and limits the behaviours that can be studied[24]. New single-objective light-sheet approaches permit rapid volumetric imaging with low photodamage and modest trade-offs in resolution, expanding the range of behaviours and animals that can be studied[21].

Another approach is to use optics to capture information from a 3D volume directly on a 2D sensor, albeit at the cost of *xy* resolution and field of view. One way to accomplish this is to tile images from different focal depths on the sensor (multifocus microscopy)[25,26]. A related strategy is light-field microscopy, which uses microlens arrays to preserve 3D information in the emitted rays to enable computational reconstruction of volumes from sensor data[27–31].

Challenges and future directions. Whatever the optical hardware, whole-brain imaging also requires complete optical access to every neuron inside a behaving animal. The development of non-invasive strategies to image brains of animals with opaque cuticles without surgery will allow cleaner access to behaviourally relevant brain activity[32]. Expanding the toolbox of techniques for whole-brain recording will increase the numbers of animals and behaviours that can be studied with systems-level approaches.

Each microscopy technique offers a different ratio of the speed–resolution trade-off. Combining techniques such as spinning-disk confocal microscopy and light-field microscopy[30], or two-photon with light-sheet microscopy[33], or incorporating deep-learning techniques for resolution enhancement[31] can partially alleviate the speed–resolution trade-off. Whereas the small size of *C. elegans* enables functional whole-brain imaging in freely moving animals at high speeds and at single-cell resolution[34,35], and recent work in *Drosophila* has enabled high-speed recording of flies walking on a ball with single-cell resolution[36], for larger organisms it remains a challenge to develop microscopy systems capable of recording functional whole-brain data sets with cellular resolution at speeds that match the multiple timescales of neural and behavioural dynamics.

As the ability to perform whole-brain imaging during behaviour increases, so does the problem of dealing with the enormous amount of data that it rapidly generates. Microscopes measuring whole-brain neuronal activity generate raw image data at 1 GB s^{-1} or more. These data must be reduced into compact time traces corresponding to the activity of discrete neurons or brain regions. Segregating the activity of individual neurons is challenging when neurons and nerve fibres are densely packed in a brain volume or when neurons move relative to one another because of animal self-movement.

Another challenge for whole-brain imaging in freely moving animals is the improvement of tracking algorithms. *C. elegans* and zebrafish display movements of high complexity[37] and tracking has largely relied on proportional error-correction control software[29,34,35,38]. This method compensates for changes in position but does not compensate for the deformation and changes in brain orientation. In the case of *C. elegans*, the brain deforms as the worm moves, making it difficult to track the identity of the neurons being recorded over time. Recent studies, train deep neural networks to recognize the configurations the brain adopts in different worm postures. This approach enables tracking neurons with ~74% accuracy[39,40]. In larval zebrafish, the brain does not undergo significant deformation during free swimming behaviour, and data analysis relies on mapping the recorded brains onto a reference brain atlas that enables near single-cell resolution alignment[38,41]. Nonetheless, tracking the identity of neurons in different fish remains an unresolved challenge.

Body posture dynamics in *Drosophila* involves the use of six legs and a pair of wings, making posture dynamics segmentation a complex computational challenge. In recent years, deep neural network techniques for pose estimation[42–44] and unsupervised techniques for body position dynamics[45] have enabled the development of predictive models of behaviour with improved spatial and temporal resolution[37]. Incorporating these developments in animal pose estimation and predictive models of behaviour[46] into tracking control algorithms will substantially improve the throughput and quality of whole-brain data sets in behaving flies.

Table 1 | **Model organisms for whole-brain imaging during natural behaviour**

Species	Number of neurons	Behaviours studied	Experimental access
Nematode *Caenorhabditis elegans* 	300	Crawling; escape response; mating	Single-neuron resolution; identifiable neurons
Zebrafish *Danio rerio* 	10^5 (larva)	Swimming; phototaxis; prey capture	Single-neuron resolution; aligned brain atlas
Fruit fly *Drosophila melanogaster* 	10^4 (larva), 10^5 (adult)	Walking; flight; courtship; auditory responses	Single-neuron or brain region resolution; aligned brain atlas

Three model organisms

Whole-brain imaging methods were first demonstrated in immobilized animals. Brain activity could be correlated with fictive behavioural read-outs, such as the activity of muscles or command motor neurons. However, it has become possible to extract whole-brain activity from animals behaving more naturally and navigating real or virtual spaces nearly unimpeded (FIGS 1,2). We briefly review some of the unique advantages of three animals — the nematode *C. elegans*, the fruit fly *D. melanogaster* and the larval zebrafish *D. rerio* (TABLE 1) — and how whole-brain imaging has advanced understanding of their behaviour.

The nematode C. elegans. The compact nervous system of the nematode *C. elegans* is ideal for whole-brain experiments. Most of these worms are hermaphrodites and have 302 neurons with a largely stereotyped wiring diagram. Approximately 200 neurons form an anterior brain, and approximately 100 neurons form the motor

circuit[47,48]. In addition, some *C. elegans* are male and have an additional 100 sex-specific neurons in their tail, which orchestrate mating behaviour[49].

The worm brain's small size allows it to be rapidly imaged with single-cell resolution using light microscopy — either the anterior brain shared by all worms or

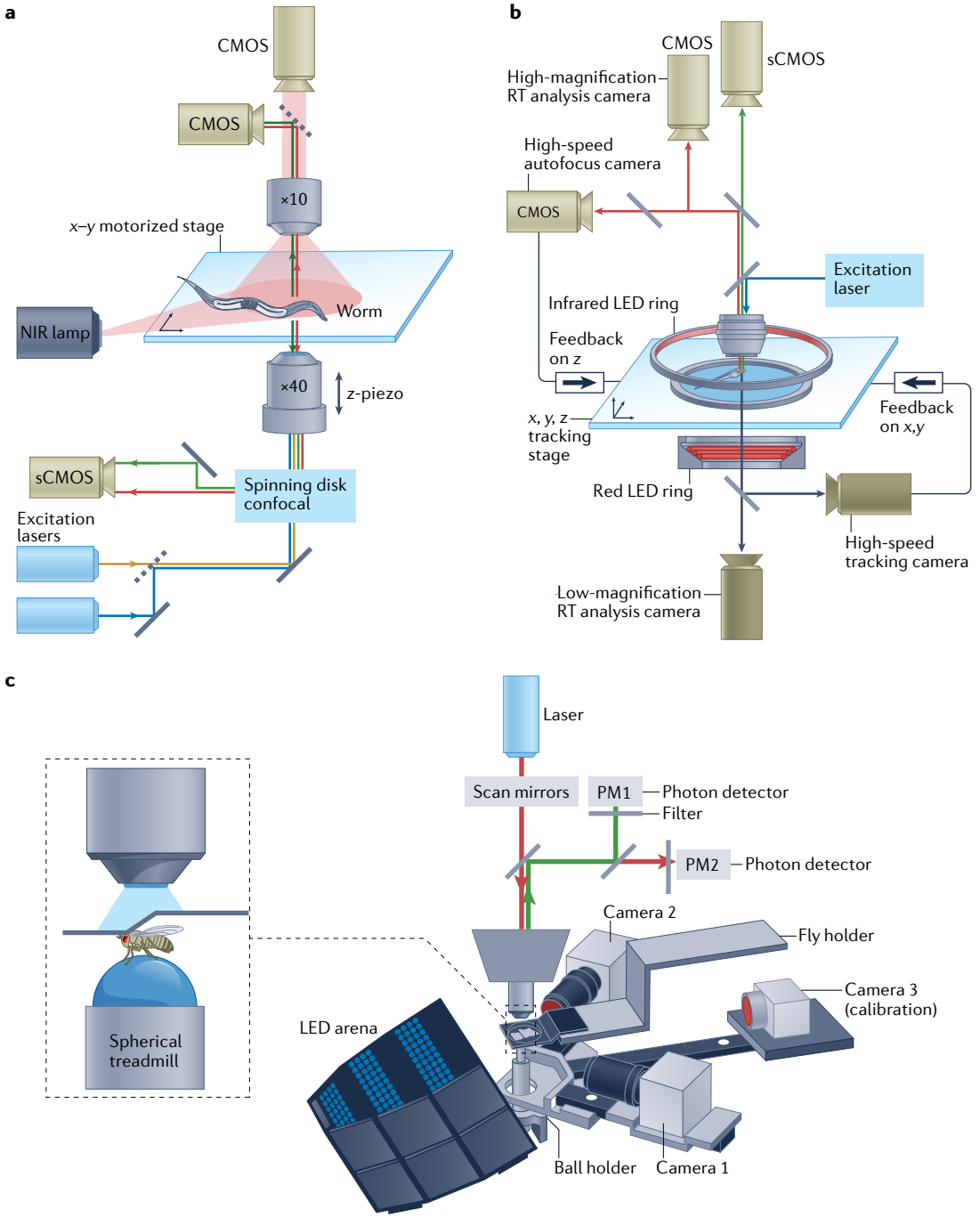


Fig. 1 | **Recording from the brains of behaving animals. a** | A *Caenorhabditis elegans* worm crawls freely on a motorized stage. A low-magnification (×10) objective captures the animal's entire body to record posture and behaviour, while a high-magnification (×40) objective records calcium activity from the animal's brain. Real-time feedback keeps the animal in the objectives' field of view. **b** | A larval zebrafish swims in a thin, water-filled chamber. A high-speed, low-magnification optical setup tracks the animal's motion, while a high-resolution light-field setup records whole-brain calcium activity. Real-time feedback in all three dimensions keeps the animal's brain in the field of view. **c** | An adult fruit fly is tethered and placed on an air-cushioned ball. A high-resolution objective allows for two-photon excitation and recording of calcium activity from the animal's head. The fly is free to walk in any direction on the ball, with low-resolution cameras recording the animal's posture and behaviour. Visual and auditory stimuli are presented to the animal while it is on the ball. CMOS, complementary metal–oxide–semiconductor; LED, light-emitting diode; NIR: near-infrared; PM: photomultiplier; RT: real-time; sCMOS: scientific complementary metal–oxide–semiconductor. Part **a** is adapted with permission from REF.[54], National Academy of Sciences. Part **b** is adapted with permission from REF.[29], CC BY 4.0. Part **c** is adapted with permission from REF.[71], Springer Nature Ltd.

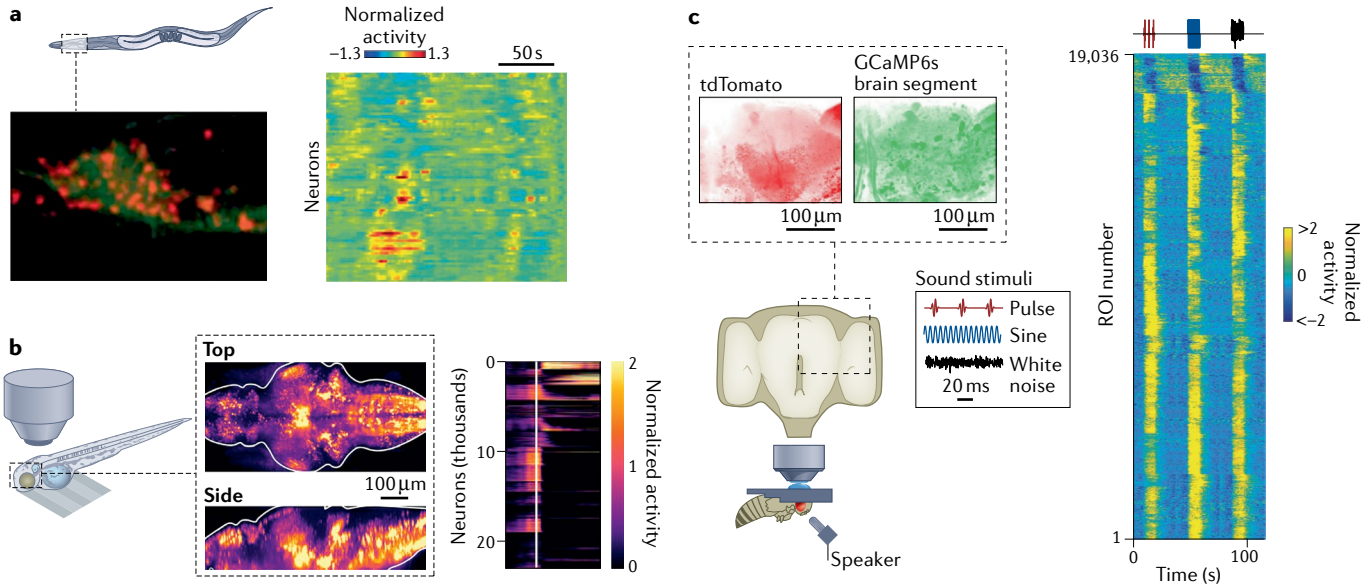


Fig. 2 | **Samples of pan-neuronal recordings in behaving animals. a** | A sample image of the brain of *Caenorhabditis elegans* (left), labelled with pan-neuronal cytosolic GCaMP6s and nuclear-localized Tag-RFP (red fluorescent protein). Normalized activity traces (right) of 84 neurons in a freely crawling worm. **b** | Top and side views of the brain of a larval zebrafish (left), labelled with GCaMP6f. Activity of segmented neurons in the brain (right) during fictive swim behaviour. **c** | Schematic of volumetric imaging of the brain of an adult *Drosophila* being presented with auditory stimuli (left). The brain is labelled with GCaMP6s and tdTomato. Responses from recorded regions of interest (ROIs) to auditory stimuli (right). Part **a** is adapted with permission from REF.[34], National Academy of Sciences. Part **b** is adapted with permission from REF.[94], Elsevier. Part **c** is adapted with permission from REF.[76], Springer Nature Ltd.

the posterior male-specific 'brain'[50]. Whole-brain imaging was pioneered in immobilized worms, in which it was discovered that even without external stimulus, a large proportion of the brain's neurons engage in coordinated activity. When this whole-brain activity is projected onto a low-dimensional representation, brain dynamics follow a cyclical trajectory[51]. Portions of the cycle correspond to the activity of pre-motor interneurons known to be associated with locomotion direction, allowing epochs of fictive forward and backward movement to be inferred in stationary animals. The stereotyped brain-wide activity patterns for forward/backward behavioural states have been interpreted to represent global commands that account for the majority of the variance in neural dynamics.

Forward and backward locomotion are slowly changing behavioural states, but within each state, muscle activity occurs on faster timescales to drive rapid exploratory head bending and rhythmic body undulation[52]. Despite the difference in timescales, the neurons that drive these movements are directly modulated by other neurons with slowly changing activity that are correlated with forward/backward behavioural state changes. The activity and cross-modulation of neurons across a hierarchy of timescales occur in both moving and immobilized worms. Nested activity dynamics across timescales appears to be an organizing principle of the brain circuit, both during unrestrained and fictive behaviour[53].

Comparing whole-brain dynamics in immobilized animals to independent behavioural experiments in moving animals can illuminate correlations between circuit activity and behaviour. To more carefully dissect the mechanisms in whole-brain dynamics that produce behaviour, brain and behavioural dynamics can be studied at the same time in the same animal. Improvements in volumetric imaging speed and single-neuron tracking now enable whole-brain recording in freely moving worms[34,54] (FIG. 2a). As observed in immobilized worms, large numbers of neurons in the brain are correlated with forward and backward movement. In freely moving worms, however, substantial diversity in brain dynamics is observed, with activity often correlated with additional quantifiable parameters of worm movement such as velocity and curvature. Reliably decoding these behavioural details from brain-wide activity requires large numbers of neurons, hinting at a more subtle and distributed neural code for the full dynamics of worm behaviour[55]. Moreover, the correlation structure between certain pairs of neurons changes markedly when freely moving worms are immobilized. Thus, the neural dynamics of fictive behaviours in immobilized worms are measurably different from the corresponding neural dynamics in unrestrained worms, an important caveat when trying to understand a natural behaviour by studying immobilized animals.

In general, whole-brain recording studies face the challenge of matching neurons between animals. In *C. elegans*, every neuron follows a stereotyped lineage across development and has a largely stereotyped connectivity to other neurons. In principle, one should be able to compare whole-brain activity of different animals by aligning the activities of the same neurons. However, animal-to-animal variability in the relative positions of cell bodies makes the neuronal identities

difficult to determine. To identify neurons, one needs additional cell-specific information. In *C. elegans*, substantial knowledge of gene expression patterns provides a means of adding identifiers to neurons. Labelling a cell or group of cells of interest with a fluorescent protein with an emission spectrum orthogonal to that of the calcium sensor allows specific cells to be tagged and identified. Recently, a combinatorial method of adding many fluorescent labels of different colours was applied to the entire nervous system, aiding neuron identification during whole-brain imaging[56].

The tail of the male *C. elegans* contains a separate brain for mating with hermaphrodites[57]. Male mating behaviour is a complex multistep behaviour composed of numerous component behaviours that occur in different stimulus-evoked sequences from event to event. The entire mating circuit in the male tail can be imaged continuously while the male performs all the steps of mating behaviour. The full diversity of stimulus and motor patterns that occur during mating behaviour are represented in a similarly diverse set of neuronal activity patterns in the male tail. The unique activity patterns exhibited by many neurons with respect to the entire trajectory of the mating behaviour aid neuronal identification when performing whole-brain imaging. Many neurons contribute to multiple sub-behaviours in different ways, leading to different correlation patterns throughout the circuit in different contexts. Functional correlations between neurons are not fixed, but explicitly depend on context and behavioural state[58]. Nevertheless, many quantitative aspects of male mating behaviour can be decoded from brain-wide activity pattern.

Whole-brain imaging promises to shed light on many aspects of worm behaviour, but a major hurdle is data analysis. Extracting signals with minimal motion artifacts is challenging in an animal in which the brain itself deforms during normal locomotion[35,59–61]. As more behaviours are studied for long periods of time (timescale of tens of minutes or even hours), data analysis needs to become increasingly automated without losing the reliability and accuracy of manual annotation (as discussed in the section on Computational methods).

Another challenge is that, in some cases, different calcium activity patterns are encoded in different parts of the same neuron. To more easily separate traces from neighbouring cells, most whole-brain imaging studies have used nuclear markers of calcium dynamics. Doing so creates a well-separated constellation of discrete imaging volumes for all neurons, but misses computationally relevant calcium dynamics that in many neurons may occur only in the nerve fibres and processes[62–64]. Whole-brain imaging with comprehensive nerve fibre segmentation imaging in the small worm brain is difficult to imagine with current methods. However, in an animal that encodes the full range of its complex behaviours in only hundreds of neurons, the computing power of single cells should not be underestimated. The sophistication of single cells in *C. elegans* is demonstrated in its motor circuit. In larger animals, networks of spinal cord neurons give rise to rhythmic and organized movements[65–68]. In *C. elegans*, single motor neuron types encode the properties of networks of cells found in larger animals[52,69]. Careful analyses of spatio-temporal properties of specific neuron classes will continue to play a vital role, even with the availability of whole-brain approaches.

The fruit fly D. melanogaster. Since the advent of optical methods for recording brain activity using transgenic animals, the fruit fly *Drosophila* has been a widely used model for systems neuroscience: from its larval stage (with about 10,000 neurons) to its adult stage (with about 100,000 neurons)[70]. These two life stages have different behavioural repertoires. Larval behaviour primarily consists of foraging for food and avoiding threats, whereas the adult fly exhibits a wider range of complex behaviours. The adult integrates visual, auditory and chemosensory cues when flying and walking, and when engaging in social behaviours such as courtship, mating and aggression.

Whole-brain imaging in adult *Drosophila* is possible with either light-field microscopy or fast volumetric two-photon microscopy. To visualize the entire brain with cellular resolution via imaging, the fly's brain must be exposed and its head fixed with respect to a microscope, limiting its range of motion. Nevertheless, a rich set of sensorimotor behaviours can be explored with head-fixed flies in tethered flight or walking on trackballs[71].

The relatively large size of the adult *Drosophila* brain makes it difficult to record from the whole brain at once with high spatial and temporal resolution. When whole-brain recording is performed with uniformly labelled cells, the dense packing of cell bodies and neurites makes it difficult to resolve the optical signal of individual neurons. Because it is impossible to align individual neurons across animals, comparing experiments requires computational registration of recordings from different animals to a common spatial atlas[72]. Calcium dynamics in brain-wide recordings from the adult fly are often measured from the densely packed neuropil, with each imaged voxel representing the integrated activity of many neuronal fibres. These fibres — which locally receive and transmit synaptic signals and propagate activity along their lengths — generally have richer calcium dynamics than the cell bodies that are more distant from synaptic contacts. Imaging volumes rather than discrete neurons results in whole-brain activity measurements in the adult *Drosophila* with mesoscale resolution[28,73]. Such pan-neuronal recordings in the adult fly reveal common principles of whole-brain function. As in *C. elegans*, large fractions of the brain show correlated patterns of activity even in the absence of stimuli[73,74].

To isolate the activity of single cells in *Drosophila*, complementary labelling approaches are often be used. Using selective drivers of gene expression, comprehensive recordings of region-level activity can be supplemented with targeted recordings from single cells and cell types of interest. Sparse labelling strategies are another option, giving the experimenter access to a subset of neurons across the brain with single-neuron resolution[75].

Brain-wide imaging in the adult fly is now being used to perform whole-brain searches for behavioural

circuits that are less biased by expectations of where sensory and motor signals should be located. An example is the discovery of an unexpectedly widespread brain-wide response to auditory stimuli. Components of fly courtship songs evoke activity from a diverse array of brain areas in both males and females[76]. Furthermore, stimulus-evoked responses were relatively stereotyped in early mechanosensory areas of the brain, but were observed to be more variable in downstream regions. From moment to moment, different downstream brain areas respond to the same stimulus inputs. This variability is not explained by changes in the animal's instantaneous movements, suggesting that auditory information shapes, but does not alone drive, motor behaviour during courtship. Internal states also affect brain-wide activity and behaviour. In female flies, long-lasting internal states drive different brain activity patterns and behaviours in the presence of males: changes in receptivity to courtship as well as aggressive behaviours such as shoving and chasing[77].

Brain-wide imaging is also being used to uncover mechanistic principles that probably extend to whole-brain dynamics in larger animals. A study of brain-wide imaging in the adult *Drosophila* brain demonstrated the correlation between measures of metabolic activity (fluorescent indicators of intracellular molecules associated with cellular energy metabolism) and calcium activity[74]. The idea that metabolic and local neuronal activity are linked underlies fMRI in humans and other large animals, which measures metabolic activity via changes in blood flow[9]. The fact that this link is empirically validated in the fly suggests a general principle of brain physiology that seems to be shared by species separated by more than 400 million years of evolution.

Recently, using nuclear-localized GCaMP and single-oblique light-sheet (swept confocally aligned planar excitation (SCAPE)) microscopy, it has become possible to image a substantial fraction of the central brain of an adult fly (~2,000 of the 30,000 neurons in the central brain) at single-neuron resolution as it walks on a trackball[36]. Thousands of neurons in the brain were recorded as the fly performed a number of behaviours, including running, grooming and flailing. These data revealed populations of neurons correlated to behaviour over multiple timescales, from seconds to minutes. Different behaviours were coupled to distinct patterns of brain-wide activity, with some behaviours engaging the whole brain more strongly than others. Although large fractions of the brain appeared to have activity correlated with behaviour, the uncorrelated portions of the brain had high-dimensional activity. These data show that brain-wide neural activity consists of a combination of localized and broadly distributed components.

As in *C. elegans*, it is likely that when recording from neuronal nuclei alone, many signals in the neuronal processes are missed. Despite this caveat, the ability to record from thousands of neurons simultaneously in the fly brain represents a considerable advance. These results also highlight a key advantage of whole-brain approaches: the ability to contextualize the activity of a single circuit within a larger network.

It is also possible to capture the activity of the whole central nervous system of an immobilized *Drosophila* larva with light-sheet microscopy[78]. Whole-brain recording in a crawling *Drosophila* larva is harder because of the movements and deformations of the brain in freely crawling animals[79]. However, the fictive motor behaviours of a brain that was surgically removed from a larva's body could be inferred from the activity of its ventral nerve cord in a whole-brain imaging study using light-sheet microscopy[78]. Two-photon tracking microscopy and single-objective light microscopy have been used to follow the activity and movements of small numbers of neurons in the motor circuit of freely moving larvae[22]. As these tracking techniques improve in spatial and temporal resolution, they are likely to extend to larger circuits for behaviour in the unrestrained larva.

The larval zebrafish D. rerio. There is a vertebrate model organism that shares the relatively small size, optical accessibility and well-developed genetic toolbox of flies and worms. The larval zebrafish (*D. rerio*) has about 100,000 neurons[80] and performs a large variety of stimulus-evoked navigational behaviours. These include hunting and prey capture, as well as threat avoidance[81]. Its brain layout has strong homologies to mammalian brains (for instance, it has a bona fide cerebellum and hypothalamus), making it a good candidate for cross-species studies[82]. However, its many neurons make it difficult to identify and compare the same labelled neurons from animal to animal. Functional analysis of whole-brain imaging focuses on identifying spatial regions of the brain with coherent activity patterns aligned to a spatial brain atlas. The relatively stereotyped overall topology of the zebrafish brain aids alignment across individuals, allowing brain maps to be compared for different animals and different experiments with near cellular resolution[41,83].

The calcium activity of the entire brain of an immobilized larval zebrafish was first recorded with single-neuron resolution using light-sheet microscopy[84]. Even in this immobilized larva, correlated activity patterns were observed in large numbers of neurons across brain regions, and cyclic activity was observed on multiple timescales in different neurons. Since then, comprehensive recording with cellular resolution has been used to study a number of sensorimotor behaviours in immobilized and semi-immobilized animals[85]. One way to decode the motor behaviour of an immobilized fish is to record the electrical activity of motor nerves in its tail during whole-brain imaging[86]. Another way is to immobilize only the head for whole-brain imaging while monitoring the free movements of the tail. Thus, a complete map of neurons and brain areas involved in various sensory to motor transformations can be obtained. More recent studies have mapped brain-wide circuits for thermosensory and optomotor responses, demonstrating the progressive computations that integrate separate sensory streams — such as separate images presented to the left and right eye, or the detection of warming, cooling and absolute temperature — into purposeful motor decisions[87,88]. Such neurons that neither strongly correlate to individual sensory inputs nor to motor outputs

represent convergence neurons that carry out intermediate steps in information processing and non-reflexive decision-making[89].

For example, the zebrafish larva has a strong and innate optomotor response that allows it to orient when it sees a moving environment. But when zebrafish are presented with images of dots that move randomly with a slight bias, they accumulate and integrate motion evidence over time before deciding in which direction the dots are moving[90,91]. The zebrafish larva also performs memory-dependent behaviours including operant conditioning[92,93]. When swimming does not result in perceived movement, fish will gradually change their willingness to perform swim bouts[94]. As the larva gradually changes its decision-making, functional correlations in a distributed brain-wide network also change. These functional changes predict the outcome of decisions and point to the distributed nature of decision-making throughout the brain[94–96] (FIG. 2b).

Like most other animals, zebrafish larva exhibit sustained behavioural states that affect brain activity. For example, brain-wide imaging has been used in the zebrafish larva to uncover sleep signatures that resemble slow-wave sleep and rapid eye movement (REM) sleep in mammals[97]. These sleep states have the same dependence on hormone signalling as the homologous states in mammals, pointing to conserved principles in the brain-wide organization of sleep.

Behavioural states in active fish can only be discerned if the fish are allowed to exhibit behaviour. One way to elicit purposeful behaviour from a fish is to close the loop between perceived motor action and an applied stimulus to effectively create a virtual reality environment that can be explored by an immobilized fish. In a study of zebrafish larvae navigating a virtual reality environment, normal exploratory behaviour was observed. However, when the system was switched to 'open loop', swim commands no longer correlated to perceived self-motion, and the fish begin swimming intensely for a period, before entering a state of futility-induced passivity[94]. Whole-brain imaging revealed the corresponding distinct brain states, and the discovery of glial cells that accumulate evidence of futility and ultimately trigger the change in behavioural state. Internal state transitions after prolonged behavioural challenges have also been demonstrated at the level of brain-wide circuits. Whole-brain imaging with prolonged behavioural challenge uncovered the progressive activation of neurons in the habenula, a brain area that controls other circuits that regulate passivity[98].

Functional whole-brain imaging studies in larval zebrafish have also enabled the discovery of neural populations with functional roles that are conserved in other vertebrates. By combining whole-brain activity with cell-type-specific markers, whole-brain imaging uncovered a variety of neuromodulatory cell types that are correlated with the animal's internal states[99]. Remarkably, homologous neuromodulatory cells in the mouse exhibited similar state-dependent dynamics to the larva, underscoring the generalization of principles learned from whole-brain imaging in small, accessible model animals.

Many complex behaviours and behavioural states only occur in unfettered animals. Certain forms of environmental feedback, such as proprioceptive or vestibular cues, cannot easily be replicated in virtual reality. One recent study of the vestibular response in an immobilized zebrafish larva was accomplished with a specialized whole-brain imaging system that rotated in its entirety[100]. Complex naturalistic behaviours, such as hunting, can only be studied in freely moving animals. The predation of *Paramecia* by zebrafish larvae is a multicomponent behaviour consisting of visual search, pursuit and prey capture. Hunting requires rapid sensory processing, motor feedback, and fast context-dependent decision-making to continue or abort a pursuit. High-speed whole-brain imaging with microscopes that track freely moving larvae has identified brain regions activated during prey capture[29]. Recordings from freely swimming zebrafish foraging for *Paramecia* have revealed transitions between distinct brain states for exploratory locomotion and for hunting, and identified the network of neurons that trigger this transition[101].

Whole-brain structural imaging

The functional imaging approaches described above provide a means of quantifying the activity of the whole brains of diverse species. The small size of the animals reviewed here is also an advantage when carrying out structural imaging, acquiring the detailed synaptic connectivity of their entire nervous systems. Determining the 'wiring diagram' (connectome) of the brain through structural imaging enables direct comparisons between functional activity data and neural anatomy. Connectomes thus place important constraints on the correlation structure of brain-wide neural activity. Connectomics requires serial-section electron microscopy — the only imaging modality with the throughput and resolving power necessary to reconstruct complete synaptic circuits.

The first near-complete synapse-level map of an entire nervous system was obtained in *C. elegans*, a heroic feat with the methodology available in the 1980s[47]. An analysis of a complete circuit for behaviour directly emerged from this connectome. Systematic laser ablation and behavioural analysis was used to map the circuit for harsh touch sensitivity — a feedforward reflex that allows the worm to avoid anterior or posterior touches by rapid backward or forward movement — from sensory neurons to interneurons to motor neurons[102]. Since this early achievement, the connectome has provided an invaluable resource for mapping behaviour to circuits in *C. elegans*. A larger challenge is to use the connectome to understand whole-brain activity patterns.

One approach to using whole-brain connectomes is to compare animals with connectomes that have informative differences. The low throughput of whole-brain connectomics precludes doing so on a large scale for most animals. Comparative connectomics, however, has begun with the nematode *C. elegans*. The connectome has been mapped for an isogenic population of nematodes across development at different time points from birth to adulthood[48]. Substantial remodelling of

synaptic circuits that is directed by a number of organizing principles and brain-wide patterns occurs during development. The changing connectome of the growing worm is likely to underlie changes in whole-brain dynamics and behaviour that accompany its maturation. Brain-wide imaging applied to the developing worm may reveal the effect of anatomical maturation on circuit dynamics.

Connectomes of larger animals are also being reconstructed. Substantial portions of the connectome of an entire *Drosophila* larval brain have been mapped, providing insights into its circuits for sensory processing, decision-making, learning and memory, and motor control[103,104]. The synapse-level connectome of an adult *Drosophila* hemibrain has now been completed, and additional connectome maps are underway[70,105,106].

The connectome of the adult *Drosophila* has been used to assess brain-wide functional connectivity. The pattern of resting-state functional correlations in brain-wide calcium activity has been shown to reflect the coarse-grained structural connectivity of the fly brain (as inferred from the full anatomical wiring diagram). A similar relationship between functional and mesoscale structural connectivity has been observed in the mammalian brain, underscoring the role of synaptic connections in shaping brain-wide activity patterns across species[107].

Structural studies are underway in the brain of the larval zebrafish. Light microscopy and the integrated analysis of a large panel of sparsely labelled transgenic fish has been used to build a comprehensive atlas of the brain with single-cell resolution[83]. Serial-section electron microscopy, albeit at lower resolution than needed for individual synapses, has been used to reconstruct the morphology of all cells and fibres in the brain[108]. With high-resolution imaging and automated analysis, complete maps of the zebrafish brain with full synaptic resolution are forthcoming[109,110].

By itself, the connectome is not sufficient to understand brain-wide dynamics. As studies of brain-wide activity repeatedly show — whether in worms, flies or fish — the same connectome can give rise to functional correlations between neurons and across brain regions that change markedly with environmental context and behavioural state. In *C. elegans*, the wiring diagram is largely stable across isogenic individuals that exhibit the same behaviours, implying functional relevance for shared wiring[48]. However, the computational properties of the brain are encoded in both its synaptic and non-synaptic pathways of communication. These pathways span spatial scales from microcircuits to the whole nervous system, and temporal scales from seconds to animal lifetimes. Connectomes, when combined with whole-brain activity patterns at the cellular and synaptic level, will be essential for modelling brain activity.

Computational tools for neural and behavioural data

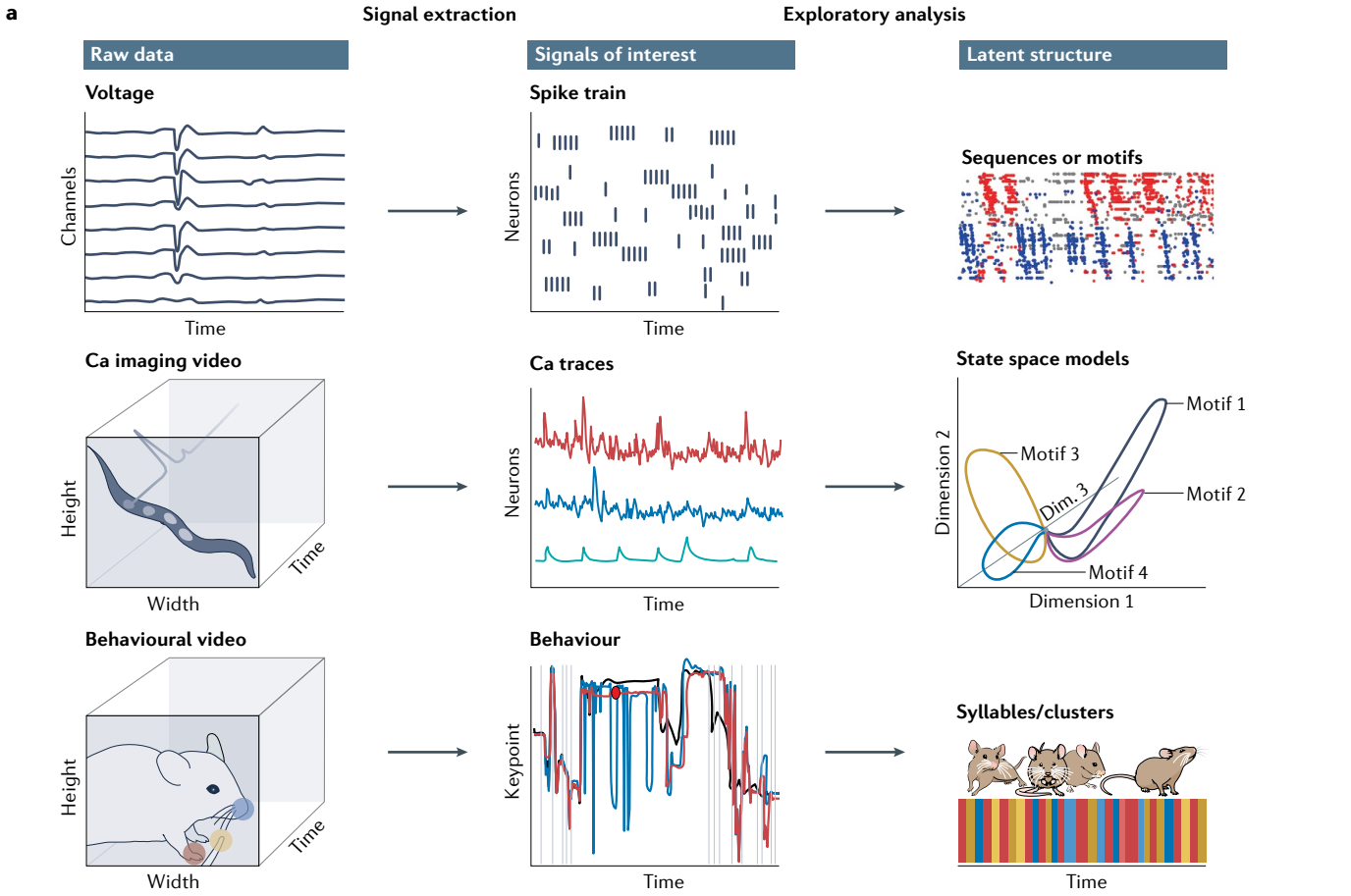
Emerging methods for high-throughput connectomics, whole-brain functional imaging and behavioural quantification are generating enormous data sets (FIG. 3). There is a pressing need for computational and statistical methods to aid in preprocessing, exploring, integrating and ultimately understanding these data. Advances are being made at each stage of analysis, but much work must be done to realize the potential of modern recording technologies and the data sets they produce.

The most immediate problem is to extract biological signals of interest from the raw data (FIG. 3a). In the experimental setups described above, a common first step is to track neurons in a video of a moving animal and estimate the calcium fluorescence in each cell over time[111–113]. In *C. elegans*, for example, the tracking problem is complicated by the fact that cells may come and go from the field of view, and their relative positions may change as the animal's body compresses and expands during movement. A variety of methods approach this problem with machine learning techniques[35,59–61,114] and experimental techniques for multicolour fluorescence imaging[56]. Machine learning is also accelerating behavioural analysis and connectomics. Markerless tracking algorithms for identifying key points of interest on an animal's body — like the centre-line of the worm, the eyes and tail of a larval zebrafish, or the legs, body and eyes of a fruit fly — have seen considerable advances in recent years[43,115–118]. These methods transfer highly tuned convolutional neural networks for human pose estimation to the animal setting with relatively little additional training. Deep learning has also been key to automatically tracing neural tissue in serial electron microscopy images for connectomics[48,70,105,108,119]. With these advances, it is now possible to measure neural and behavioural data with high resolution and to trace the neural circuits that give rise to this activity and drive motor output.

There are two approaches for gaining understanding from these large-scale neural, behavioural and connectomic data sets, once the preprocessing challenges have been surmounted. One approach is bottom-up, looking for simple, recurring patterns in the data that demand theoretical justification; the other is top-down, positing a normative theory of neural computation and hypothesizing a biological mechanism that could carry it out. These are complementary endeavours that ideally will meet in the middle[120].

Bottom-up approaches, also known as exploratory analyses[121], aid in visualizing high-dimensional data and, it is hoped, discovering unexpected structure therein (FIG. 3a). Dimensionality reduction techniques, such as clustering, principal components analysis, nonlinear manifold learning methods and dynamical systems models, are widely used in neuroscience[122]. Such techniques are used to identify stereotyped patterns of behaviour[45,123], model the temporal dynamics of such patterns[124,125] and relate neural activity to behaviour[126,127]. For example, in *C. elegans* these analyses have been used to determine that immobilization alters brain-wide neural dynamics and its correlation structure[55].

Advances in machine learning continue to expand this toolkit, offering new techniques for finding low-dimensional structure in neural and behavioural data. For example, probabilistic state-space models summarize high-dimensional time series data in terms of simpler latent 'states' and a dynamical system that governs



how states change over time[128,129]. Combined with neural networks or Gaussian processes, these approaches can find states that lie on a nonlinear manifold, or states that evolve according to nonlinear dynamics. Such methods underlie many techniques for modelling neural and behavioural time series[130–139]. One approach for using machine learning methods to learn about neural computation is to use nonlinear dynamical systems theory to characterize the learned dynamics in terms of linearizations around their fixed points[140].

Fig. 3 | **Computational methods for neural and behavioural analysis. a** | The first challenge is to develop statistical methods to extract biological signals of interest from raw data. Examples include extracting the times of action potentials ('spikes') from extracellular voltage recordings, demixing and deconvolving calcium fluorescence traces, or tracking body parts in videos. Computational models for exploratory analysis aim to reveal simplifying structure in high-dimensional signals, such as repeated sequences of spikes, low-dimensional trajectories of neural activity, or clusters of stereotyped behaviours. **b** | Top-down analyses hypothesize an algorithm and circuit implementation to solve a computational problem, such as tracking heading given visual inputs and proprioceptive feedback. **c** | Rather than hand-tuning an algorithm and circuit, task-based modelling learns a circuit to solve a particular computation by minimizing a loss function. **d** | Top-down models make predictions about neural activity that can be tested against measured data; task-based modelling offers an indirect way of making testable predictions of neural activity. Part **a** is adapted with permission from REF.[154], CC BY 4.0.

What have these bottom-up approaches shown? In the study of motor cortical dynamics during reaching, a context in which many of these methods were pioneered, dynamical systems models have shown how complex single-cell tuning curves can be explained by a few population-level states[141]. In immobilized *C. elegans*, these approaches have shown how whole-brain activity can be characterized by a low-dimensional dynamical system with approximately linear dynamics corresponding to behaviours like forward crawling, reversals and turns[51]. As we look toward whole-brain recordings in more naturalistic behaviour, state-space models offer a means of relating neural and behavioural data in terms of low-dimensional latent states that are easier to understand.

By contrast, top-down approaches start with a theoretical model of how a particular computation could be carried out, and from there derive predictions about neural and/or behavioural data (FIG. 3b). For example, theoretical neuroscientists hypothesized that an idealized neural circuit called a ring attractor could maintain an internal estimate of an animal's heading direction[142,143]. In the model, there is a population of neurons with each neuron tuned to a particular heading: its firing rate is highest when the animal is facing in its preferred direction. The population of neurons produces a 'bump' of activity in the subset of similarly tuned neurons, through a balance of excitatory and inhibitory synapses. Sensory cues and proprioceptive feedback provide external inputs to the circuit, causing the bump to move in accordance with the animal's heading. Experiments have identified such a circuit in the *Drosophila* central complex[144], and, remarkably, the cells are physically arranged in a ring, just as the theory predicted.

Rarely are theoretical models borne out so nicely in practice. Many of the brain's computations are too complex for closed-form, theoretical solutions. Instead, computational neuroscientists have recently turned to 'task-based' modelling, which leverages artificial intelligence and deep learning[145–148]. The idea is to model an artificial agent performing the same computation (that is, task) as the animal, but using an artificial neural network in place of a biological one (FIG. 3c). Rather than solving for the optimal artificial network weights analytically, task-based modelling uses stochastic gradient descent to search for an approximately optimal configuration. The trained artificial agent offers a reference point for studies of biological nervous systems. In particular, the 'neural activity' of the artificial agent (that is, the activation of units in its artificial neural network) offers a prediction of neural activity in the biological organism (FIG. 3d). The key idea is that it is often easier to identify the computational problem and the architectural constraints than it is to solve for the theoretically optimal solution, and deep learning algorithms can solve the hard problem of finding optimal network weights for a given task. In this sense, task-based modelling offers a new approach to connecting top-down theories of computation to complex neural, behavioural and connectomics data.

Physics-based theoretical frameworks to merge levels of neural computation

The problem of understanding brains and behaviour is naturally exciting for physicists. The technical demands of experiments and the challenges of understanding large and complex data sets have progressed to the point that many whole-brain studies require collaboration between experimentalists and theorists in neuroscience and biophysics. We believe the same relationship between theory and experiment that characterizes many areas of physics will advance the field of whole-brain imaging. Theorists are now making useful and interesting predictions, and experimentalists can test them using the growing toolbox of molecular, cellular and structural perturbations available in genetically accessible model organisms. In this section, we describe areas where experimental and theoretical physicists can help to move the field forward, either with technological advances or mathematical modelling.

Understanding the way in which high-level computational features of brain processing such as decision-making algorithms, sensorimotor transformations and internal state trajectories emerge from the low-level activity or molecular properties of individual neurons requires the development of theoretical and computational tools that span top-down and bottom-up modelling approaches.

Physics has long navigated different levels of abstraction of natural phenomena. In non-living matter, theoretical approaches have established satisfactory descriptions of behaviour from the level of sub-atomic particles to that of entire galaxies. In living matter, physics has also succeeded in bridging different levels through coarse-graining. For example, in the study of bacterial chemotaxis[149], models that describe how operon structure determines gene expression have been incorporated into higher-level models that describe the behaviour of populations of freely swimming bacteria[150]. This multiscale theoretical approach merges physics-based models of molecular networks with physics-based models of random walks. It led to understanding the way in which correlation structure in gene expression can shape the distribution of behaviours in a bacterial population, and the manner in which this determines environmental fitness[151].

In neuroscience, physics-based models exist at many scales, from descriptions of ion channels and detailed Hodgkin and Huxley models of neurons and small

circuits[119,152] to maximum entropy models of whole-brain activity[153] and phenomenological models of decision-making and behaviour[37]. Theoretical efforts to understand higher-level brain function from whole-brain activity and connectomics should not be limited to dynamical systems that transform neural dynamics into behavioural dynamics. They should also incorporate levels of abstraction where the contribution of circuit properties at multiple scales — such as network motifs, control algorithms, relative timescale constraints and weak linkage — can be tested. This challenge could be tackled, for example, by starting with computational multiscale agent-based models that incorporate different scales of abstraction and then moving to analytical models that capture relevant phenomena in the range of scales and parameters that are relevant to a specific scientific question.

Outlook

Whole-brain imaging was made possible by technological advances in optics, genetics, fluorescent sensors and computational image analysis. The resulting whole-brain data sets have allowed new theoretical frameworks to be compared against measured data. Looking forward, we hope that continued advancements in both experimental and theoretical methods will enhance our understanding of brain-wide computation.

The complex behaviours exhibited by worms, flies and fish are analogous to behaviours studied in larger animals. In these larger animals, however, it is only possible to study these behaviours with more compartmentalized approaches. The identification of common principles in brain dynamics and behaviour in these genetically tractable small model organisms are likely to represent principles that are widely shared across the animal kingdom.

Neuroscience has historically been constrained by the available technologies to reductionist approaches to understanding behaviour, recording from small numbers of neurons in controlled settings. Conversely, ethology — the quantitative study of behaviour — has relied on careful observations to study natural animal behaviour. Determining the neural basis of animal behaviour has been a long-standing interest of both fields. However, because behaviour often engages widely distributed brain circuits, until recently it has not been possible to simultaneously capture behaviour and high-dimensional neural activity[46]. Advances in physics, biotechnology and computer science have allowed this gap to be bridged. Whole-brain approaches to brain dynamics and structure are now opening a new and interdisciplinary field: studying the neural basis of natural behaviour.

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1. Dupre, C. & Yuste, R. Non-overlapping neural networks in *Hydra vulgaris*. *Curr. Biol.* **27**, 1085–1097 (2017).
2. Chen, J. L., Andermann, M. L., Keck, T., Xu, N. L. & Ziv, Y. Imaging neuronal populations in behaving rodents: paradigms for studying neural circuits underlying behavior in the mammalian cortex. *J. Neurosci.* **33**, 17631–17640 (2013).
3. Hong, G. & Lieber, C. M. Novel electrode technologies for neural recordings. *Nat. Rev. Neurosci.* **20**, 330–345 (2019).
4. Allen, W. E. Thirst regulates motivated behavior through modulation of brainwide neural population dynamics. *Science* **364**, 253 (2019).
5. Allen, W. E. et al. Global representations of goal-directed behavior in distinct cell types of mouse neocortex. *Neuron* **94**, 891–907 (2017).
6. Stringer, C. et al. Spontaneous behaviors drive multidimensional, brainwide activity. *Science* **364**, 255 (2019).
7. Steinmetz, N. A. et al. Neuropixels 2.0: a miniaturized high-density probe for stable, long-term brain recordings. *Science* **372**, eabf4588 (2021).
8. Steinmetz, N. A., Koch, C., Harris, K. D. & Carandini, M. Challenges and opportunities for large-scale electrophysiology with neuropixels probes. *Curr. Opin. Neurobiol.* **50**, 92–100 (2018).
9. DeYoe, E. A., Bandettini, P., Neitz, J., Miller, D. & Winans, P. Functional magnetic resonance imaging (FMRI) of the human brain. *J. Neurosci. Methods* **54**, 171–187 (1994).
10. Lee, J. H. et al. Global and local FMRI signals driven by neurons defined optogenetically by type and wiring. *Nature* **465**, 788–792 (2010).
11. Calhoun, A. J. & Murthy, M. Quantifying behavior to solve sensorimotor transformations: advances from worms and flies. *Curr. Opin. Neurobiol.* **46**, 90–98 (2017).
12. Kandel, E. R., Schwartz, J. H., Jessell, T. M., Siegelbaum, S. A. & Hudspeth, A. J. *Principles of Neural Science* 5th edn (McGraw-Hill, 2013).
13. Chen, Z., Truong, T. M. & Ai, H.-w. Illuminating brain activities with fluorescent protein-based biosensors. *Chemosensors* **5**, 32 (2017).
14. Deo, C. & Lavis, L. D. Synthetic and genetically encoded fluorescent neural activity indicators. *Curr. Opin. Neurobiol.* **50**, 101–108 (2018).
15. Pal, A. & Tian, L. Imaging voltage and brain chemistry with genetically encoded sensors and modulators. *Curr. Opin. Chem. Biol.* **57**, 166–176 (2020).
16. Laviv, T. & Yasuda, R. Imaging neuronal protein signaling dynamics in vivo. *Curr. Opin. Neurobiol.* **69**, 68–75 (2021).
17. Stephens, D. & Allan, V. Light microscopy techniques for live cell imaging. *Science* **300**, 82–86 (2003).
18. Huisken, J. & Stainier, D. Y. R. Selective plane illumination microscopy techniques in developmental biology. *Development* **136**, 1963–1975 (2009).
19. Svoboda, K. & Yasuda, R. Principles of two-photon excitation microscopy and its applications to neuroscience. *Neuron* **50**, 823–839 (2006).
20. Bouchard, M. B. et al. Swept confocally-aligned planar excitation (SCAPE) microscopy for high-speed volumetric imaging of behaving organisms. *Nat. Photonics* **9**, 113–119 (2015).
21. Voleti, V. et al. Real-time volumetric microscopy of in vivo dynamics and large-scale samples with SCAPE 2.0. *Nat. Methods* **16**, 1054–1062 (2019).
22. Karagyozov, D., Mihovilovic Skanata, M., Lesar, A. & Gershow, M. Recording neural activity in unrestrained animals with three-dimensional tracking two-photon microscopy. *Cell Reports* **25**, 1371–1383 (2018).
23. Griffiths, V. A. et al. Real-time 3D movement correction for two-photon imaging in behaving animals. *Nat. Methods* **17**, 741–748 (2020).
24. Power, R. M. & Huisken, J. A guide to light-sheet fluorescence microscopy for multiscale imaging. *Nat. Methods* **14**, 360–373 (2017).
25. Prabhat, P., Ram, S., Ward, E. S. & Ober, R. J. Simultaneous imaging of different focal planes in fluorescence microscopy for the study of cellular dynamics in three dimensions. *IEEE Trans. Nanobiosci.* **3**, 237–242 (2004).
26. Abrahamsson, S. et al. Multifocus microscopy with precise color multi-phase diffractive optics applied in functional neuronal imaging.*Biomed. Opt. Express* **7**, 855–869 (2016).
27. Bimber, O. & Schedl, D. C. Light-field microscopy: a review. *J. Neurol. Neuromed.* **4**, 1–6 (2019).
28. Aimon, S. et al. Fast near-whole-brain imaging in adult *Drosophila* during responses to stimuli and behavior. *PLoS Biol.* **17**, e2006732 (2019).
29. Cong, L. et al. Rapid whole brain imaging of neural activity in freely behaving larval zebrafish (*Danio rerio*). *eLife* **6**, e28158 (2017).
30. Zhang, Z. et al. Imaging volumetric dynamics at high speed in mouse and zebrafish brain with confocal light field microscopy. *Nat. Biotechnol.* **39**, 74–83 (2021).
31. Wang, Z. et al. Real-time volumetric reconstruction of biological dynamics with light-field microscopy and deep learning. *Nat. Methods* **18**, 551–556 (2021).
32. Aragon, M. J. et al. Multiphoton imaging of neural structure and activity in *Drosophila* through the intact cuticle. *eLife* **11**, e69094 (2022).
33. Mahou, P., Vermot, J., Beaurepaire, E. & Supatto, W. Multicolor two-photon light-sheet microscopy. *Nat. Methods* **11**, 600–601 (2014).
34. Venkatachalam, V. et al. Pan-neuronal imaging in roaming *Caenorhabditis elegans*. *Proc. Natl Acad. Sci. USA* **113**, 1082–8 (2016).
35. Nguyen, J. P., Linder, A. N., Plummer, G. S., Shaevitz, J. W. & Leifer, A. M. Automatically tracking neurons in a moving and deforming brain.*PLoS Comput. Biol.* **13**, e1005517 (2017).
36. Schaffer, E. S. et al. Flygenvectors: the spatial and temporal structure of neural activity across the fly brain. Preprint at *bioRxiv* https://doi.org/10.1101/2021.09.25.461804 (2021).
37. Brown, A. E. & De Bivort, B. Ethology as a physical science. *Nat. Phys.* **14**, 653–657 (2018).
38. Kim, D. H. et al. Pan-neuronal calcium imaging with cellular resolution in freely swimming zebrafish. *Nat. Methods* **14**, 1107–1114 (2017).
39. Wu, Y. et al. Rapid detection and recognition of whole brain activity in a freely behaving *Caenorhabditis elegans*. Preprint at https://arxiv.org/abs/2109.10474 (2021).
40. Yu, X. et al. Fast deep neural correspondence for tracking and identifying neurons in *C. elegans* using semi-synthetic training. *eLife* **10**, e66410 (2021).
41. Randlett, O. et al. Whole-brain activity mapping onto a zebrafish brain atlas. *Nat. Methods* **12**, 1039–1046 (2015).
42. Pereira, T. D. et al. Fast animal pose estimation using deep neural networks. *Nat. Methods* **16**, 117–125 (2019).
43. Pereira, T. D. et al. SLEAP: multi-animal pose tracking. Preprint at *bioRxiv* https://doi.org/10.1101/2020.08.31.276246 (2020).
44. Graving, J. M. et al. DeepPoseKit, a software toolkit for fast and robust animal pose estimation using deep learning. *eLife* **8**, e47994 (2019).
45. Berman, G. J., Choi, D. M., Bialek, W. & Shaevitz, J. W. Mapping the stereotyped behaviour of freely moving fruit flies. *J. R. Soc. Interface* **11**, 20140672 (2014).

46. Datta, S. R., Anderson, D. J., Branson, K., Perona, P. & Leifer, A. Computational neuroethology: a call to action. *Neuron* **104**, 11–24 (2019).
47. White, J., Southgate, E., Thomson, J. & Brenner, S. The structure of the nervous system of the nematode *Caenorhabditis elegans*. *Phil. Trans. R Soc. Lond. B* **314**, 1–340 (1986).
48. Witvliet, D., Mulcahy, B., Mitchell, J.K. et al. Connectomes across development reveal principles of brain maturation. *Nature* **596**, 257–261 (2021).
49. Barr, M. M. & Garcia, L. R. Male mating behavior. *WormBook* https://doi.org/10.1895/wormbook.1.78.1 (2006).
50. Schrödel, T., Prevedel, R., Aumayr, K., Zimmer, M. & Vaziri, A. Brain-wide 3D imaging of neuronal activity in *Caenorhabditis elegans* with sculpted light. *Nat. Methods* **10**, 1013–1020 (2013).
51. Kato, S. et al. Global brain dynamics embed the motor command sequence of *Caenorhabditis elegans*. *Cell* **163**, 656–669 (2015).
52. Zhen, M. & Samuel, A. D. *C. elegans* locomotion: small circuits, complex functions. *Curr. Opin. Neurobiol.* **33**, 117–126 (2015).
53. Kaplan, H. S., Salazar Thula, O., Khoss, N. & Zimmer, M. Nested neuronal dynamics orchestrate a behavioral hierarchy across timescales. *Neuron* **105**, 562–576 (2020).
54. Nguyen, J. P. et al. Whole-brain calcium imaging with cellular resolution in freely behaving *Caenorhabditis elegans*. *Proc. Natl Acad. Sci. USA* **113**, 1074–81 (2016).
55. Hallinen, K. M. et al. Decoding locomotion from population neural activity in moving *C. elegans*. *eLife* **10**, e66135 (2021).
56. Yemini, E. et al. NeuroPAL: a multicolor atlas for whole-brain neuronal identification in *C. elegans*. *Cell* **184**, 272–288 (2021).
57. Sulston, J. E., Albertson, D. G. & Thomson, J. N. The *Caenorhabditis elegans* male: postembryonic development of nongonadal structures. *Dev. Biol.* **78**, 542–576 (1980).
58. Susoy, V. et al. Natural sensory context drives diverse brain-wide activity during *C. elegans mating*. *Cell* **184**, 5122–5137 (2021).
59. Nejatbakhsh, A. et al. Demixing calcium imaging data in *C. elegans* via deformable non-negative matrix factorization. *Med. Image Comput. Comput. Assist. Interv.* **12265**, 14–24 (2020).
60. Chaudhary, S., Lee, S. A., Li, Y., Patel, D. S. & Lu, H. Automated annotation of cell identities in dense cellular images. *eLife* **10**, e60321 (2021).
61. Yu, X. et al. Fast deep learning correspondence for neuron tracking and identification in *C. elegans* using synthetic training. *eLife* **10**, e66410 (2021).
62. Hendricks, M., Ha, H., Maffey, N. & Zhang, Y. Compartmentalized calcium dynamics in a *C. elegans* interneuron encode head movement. *Nature* **487**, 99–103 (2012).
63. Ouellette, M. H., Desrochers, M. J., Gheta, I., Ramos, R. & Hendricks, M. A gate-and-switch model for head orientation behaviors in *C. elegans*. *eNeuro* **5**, ENEURO.0121-18.2018 (2018).
64. Cuentas-Condori, A. et al. *C. elegans* neurons have functional dendritic spines. *eLife* **8**, e47918 (2019).
65. Mullins, O. J., Hackett, J. T., Buchanan, J. T. & Friesen, W. O. Neuronal control of swimming behavior: comparison of vertebrate and invertebrate model systems. *Prog. Neurobiol.* **93**, 244–269 (2011).
66. Kiehn, O. Development and functional organization of spinal locomotor circuits. *Curr. Opin. Neurobiol.* **21**, 100–109 (2011).
67. Rybak, I. A., Dougherty, K. J. & Shevtsova, N. A. Organization of the mammalian locomotor CPG: review of computational model and circuit architectures based on genetically identified spinal interneurons. *eNeuro* **2**, ENEURO.0069-15.2015 (2015).
68. Falgairolle, M., Puhl, J. G., Pujala, A., Liu, W. & O'Donovan, M. J. Motoneurons regulate the central pattern generator during drug-induced locomotor-like activity in the neonatal mouse. *eLife* **6**, e26622 (2017).
69. Cohen, N. & Sanders, T. Nematode locomotion: nissecting the neuronal-environmental loop. *Curr. Opin. Neurobiol.* **25**, 99–106 (2014).
70. Scheffer, L. K. et al. A connectome and analysis of the adult *Drosophila* central brain. *eLife* **9**, e57443 (2020).
71. Seelig, J. D. et al. Two-photon calcium imaging from head-fixed *Drosophila* during optomotor walking behavior. *Nat. Methods* **7**, 535–540 (2010).
72. Ito, K. et al. A systematic nomenclature for the insect brain. *Neuron* **81**, 755–765 (2014).
73. Mann, K., Gallen, C. L. & Clandinin, T. R. Whole-brain calcium imaging reveals an intrinsic functional network in *Drosophila*. *Curr. Biol.* **27**, 2389–2396 (2017).
74. Mann, K., Deny, S., Ganguli, S. et al. Coupling of activity, metabolism and behaviour across the *Drosophila* brain. *Nature* **593**, 244–248 (2021).
75. Isaacman-Beck, J. et al. SPARC enables genetic manipulation of precise proportions of cells. *Nat. Neurosci*. **23**, 1168–1175 (2020).
76. Pacheco, D. A., Thiberge, S. Y., Pnevmatikakis, E. & Murthy, M. Auditory activity is diverse and widespread throughout the central brain of *Drosophila*. *Nat. Neurosci* **24**, 93–104 (2021).
77. Deutsch, D. et al. The neural basis for a persistent internal state in *Drosophila* females. *eLife* **9**, e59502 (2020).
78. Lemon, W. C. et al. Whole-central nervous system functional imaging in larval *Drosophila*. *Nat. Commun.* **6**, 7924 (2015).
79. Sun, X. & Heckscher, E. S. Using linear agarose channels to study *Drosophila* larval crawling behavior. *J. Vis. Exp.* **2016**, 1–7 (2016).
80. Hinsch, K. & Zupanc, G. Generation and long-term persistence of new neurons in the adult zebrafish brain: a quantitative analysis. *Neuroscience* **146**, 679–696 (2007).
81. Orger, M. B. & De Polavieja, G. G. Zebrafish behavior: opportunities and challenges. *Ann. Rev. Neurosci.* **40**, 125–147 (2017).
82. Mu, Y., Narayan, S., Mensh, B. D. & Ahrens, M. B. Brain-wide, scale-wide physiology underlying behavioral flexibility in zebrafish. *Curr. Opin. Neurobiol.* **64**, 151–160 (2020).
83. Kunst, M. et al. A cellular-resolution atlas of the larval zebrafish brain. *Neuron* **103**, 21–38.e5 (2019).
84. Ahrens, M. B., Orger, M. B., Robson, D. N., Li, J. M. & Keller, P. J. Whole-brain functional imaging at cellular resolution using light-sheet microscopy. *Nat. Methods* **10**, 413–420 (2013).
85. Vanwalleghem, G. C., Ahrens, M. B. & Scott, E. K. Integrative whole-brain neuroscience in larval zebrafish. *Curr. Opin. Neurobiol.* **50**, 136–145 (2018).
86. Ahrens, M. B., Huang, K. H., Narayan, S., Mensh, B. D. & Engert, F. Two-photon calcium imaging during fictive navigation in virtual environments. *Front. Neural Circuits* **7**, 104 (2013).
87. Dunn, T. W. et al. Brain-wide mapping of neural activity controlling zebrafish exploratory locomotion. *eLife* **5**, e12741 (2016).
88. Haesemeyer, M., Robson, D. N., Li, J. M., Schier, A. F. & Engert, F. A brain-wide circuit model of heat-evoked swimming behavior in larval zebrafish. *Neuron* **98**, 817–831 (2018).
89. Lovett-Barron, M. et al. Multiple convergent hypothalamus-brainstem circuits drive defensive behavior. *Nat. Neurosci.* **23**, 959–967 (2020).
90. Bahl, A. & Engert, F. Neural circuits for evidence accumulation and decision making in larval zebrafish. *Nat. Neurosci.* **23**, 94–102 (2020).
91. Dragomir, E. I., Štih, V. & Portugues, R. Evidence accumulation during a sensorimotor decision task revealed by whole-brain imaging. *Nat. Neurosci.* **23**, 85–93 (2020).
92. Kawashima, T., Zwart, M. F., Yang, C. T., Mensh, B. D. & Ahrens, M. B. The serotonergic system tracks the outcomes of actions to mediate short-term motor learning. *Cell* **167**, 933–946.e20 (2016).
93. Lin, Q. et al. Cerebellar neurodynamics predict decision timing and outcome on the single-trial level. *Cell* **180**, 536–551.e17 (2020).
94. Mu, Y. et al. Glia accumulate evidence that actions are futile and suppress unsuccessful behavior. *Cell* **178**, 27–43 (2019).
95. Wolf, S. et al. Sensorimotor computation underlying phototaxis in zebrafish. *Nat. Commun.* **8**, 651 (2017).
96. Chen, X. et al. Brain-wide organization of neuronal activity and convergent sensorimotor transformations in larval zebrafish. *Neuron* **100**, 876–890 (2018).
97. Oikonomou, G. et al. The serotonergic raphe promote sleep in zebrafish and mice. *Neuron* **103**, 686–701.e8 (2019).
98. Andalman, A. S. et al. Neuronal dynamics regulating brain and behavioral state transitions. *Cell* **177**, 970–985.e20 (2019).
99. Lovett-Barron, M. et al. Ancestral circuits for the coordinated modulation of brain state. *Cell* **171**, 1411–1423.e17 (2017).
100. Migault, G. et al. Whole-brain calcium imaging during physiological vestibular stimulation in larval zebrafish. *Curr. Biol.* **28**, 3723–3735.e6 (2018).
101. Marques, J. C., Li, M., Schaak, D., Robson, D. N. & Li, J. M. Internal state dynamics shape brainwide activity and foraging behaviour. *Nature* **577**, 239–243 (2020).
102. Chalfie, M. et al. The neural circuit for touch sensitivity in *Caenorhabditis elegans*. *J. Neurosci.* **5**, 956–964 (1985).
103. Berck, M. E. et al. The wiring diagram of a glomerular olfactory system. *eLife* **5**, e14859 (2016).
104. Gerhard, S., Andrade, I., Fetter, R. D., Cardona, A. & Schneider-Mizell, C. M. Conserved neural circuit structure across *Drosophila* larval development revealed by comparative connectomics. *eLife* **6**, e29089 (2017).
105. Zheng, Z. et al. A complete electron microscopy volume of the brain of adult *Drosophila melanogaster*. *Cell* **174**, 730–743.e22 (2018).
106. Dorkenwald, S. et al. FlyWire: online community for whole-brain connectomics. *Nat. Methods* **19**, 119–128 (2022).
107. Turner, M. H., Mann, K. & Clandinin, T. R. The connectome predicts resting-state functional connectivity across the *Drosophila* brain. *Curr. Biol.* **31**, 2386–2394 (2021).
108. Hildebrand, D. G. C. et al. Whole-brain serial-section electron microscopy in larval zebrafish. *Nature* **545**, 345–349 (2017).
109. Lichtman, J. W. & Denk, W. The big and the small: challenges of imaging the brain's circuits. *Science* **334**, 618–623 (2011).
110. Swanson, L. W. & Lichtman, J. W. From Cajal to connectome and beyond. *Annu. Rev. Neurosci.* **39**, 197–216 (2016).
111. Pnevmatikakis, E. A. et al. Simultaneous denoising, deconvolution, and demixing of calcium imaging data. *Neuron* **89**, 285–299 (2016).
112. Zhou, P. et al. Efficient and accurate extraction of in vivo calcium signals from microendoscopic video data. *eLife* **7**, e28728 (2018).
113. Pachitariu, M., Stringer, C. & Harris, K. D. Robustness of spike deconvolution for neuronal calcium imaging. *J. Neurosci.* **38**, 7976–7985 (2018).
114. Giovannucci, A. et al. CaImAn an open source tool for scalable calcium imaging data analysis. *eLife* **8**, e38173 (2019).
115. Mathis, A. et al. DeepLabCut: markerless pose estimation of user-defined body parts with deep learning. *Nat. Neurosci.* **21**, 1281–1289 (2018).
116. Wu, A. et al. Deep graph pose: a semi-supervised deep graphical model for improved animal pose tracking. In *Adv. Neural Inf. Proc. Syst.* **33** (NeurIPS, 2020).
117. Bala, P. C. et al. Automated markerless pose estimation in freely moving macaques with openmonkeystudio. *Nat. Commun.* **11**, 4560 (2020).
118. Zhang, L., Dunn, T., Marshall, J., Olveczky, B. & Linderman, S. Animal pose estimation from video data with a hierarchical von Mises–Fisher–Gaussian model. *Proc. Machine Learning Res.* **130,** 2800–2808 (2021).
119. Abbott, L. F. et al. The mind of a mouse. *Cell* **182**, 1372–1376 (2020).
120. Linderman, S. W. & Gershman, S. J. Using computational theory to constrain statistical models of neural data. *Curr. Opin. Neurobiol.* **46**, 14–24 (2017).
121. Tukey, J. W. *Exploratory Data Analysis* Vol. 2 (Addison-Wesley, 1977).
122. Cunningham, J. P. & Yu, B. M. Dimensionality reduction for large-scale neural recordings. *Nat. Neurosci.* **17**, 1500–1509 (2014).
123. York, R. A., Carreira-Rosario, A., Giocomo, L. M. & Clandinin, T. R. Flexible analysis of animal behavior via time-resolved manifold embedding. Preprint at *bioRxiv* https://doi.org/10.1101/2020.09.30.321406 (2021).
124. Wiltschko, A. B. et al. Mapping sub-second structure in mouse behavior. *Neuron* **88**, 1121–1135 (2015).
125. Calhoun, A. J., Pillow, J. W. & Murthy, M. Unsupervised identification of the internal states that shape natural behavior. *Nat. Neurosci.* **22**, 2040–2049 (2019).
126. Markowitz, J. E. et al. The striatum organizes 3D behavior via moment-to-moment action selection. *Cell* **174**, 44–58.e17 (2018).
127. Linderman, S. W., Nichols, A. L. A., Blei, D. M., Zimmer, M. & Paninski, L. Hierarchical recurrent state space models reveal discrete and continuous dynamics of neural activity in *C. elegans*. Preprint at *bioRxiv* https://doi.org/10.1101/621540 (2019).
128. Paninski, L. et al. A new look at state-space models for neural data. *J. Comput. Neurosci.* **29**, 107–126 (2010).

129. Murphy, K. P. *Machine Learning: A Probabilistic Perspective* Ch. 17–18 (MIT Press, 2012).
130. Eyjolfsdottir, E., Branson, K., Yue, Y. & Perona, P. Learning recurrent representations for hierarchical behavior modeling. Preprint at https://arxiv.org/abs/1611.00094 (2016).
131. Zhao, Y. & Park, I. M. Variational latent Gaussian process for recovering single-trial dynamics from population spike trains. *Neural Comput.* **29**, 1293–1316 (2017).
132. Linderman, S. W. et al. Bayesian learning and inference in recurrent switching linear dynamical systems. *PMLR* **54**, 914–922 (2017).
133. Pandarinath, C. et al. Inferring single-trial neural population dynamics using sequential auto-encoders. *Nat. Methods* **15**, 805–815 (2018).
134. Hernandez, D. et al. A novel variational family for hidden nonlinear Markov models. Preprint at https://arxiv.org/abs/1811.02459 (2018).
135. Duncker, L., Bohner, G., Boussard, J. & Sahani, M. Learning interpretable continuous-time models of latent stochastic dynamical systems. Preprint at https://arxiv.org/abs/1902.04420 (2019).
136. Batty, E. et al. BehaveNet: nonlinear embedding and Bayesian neural decoding of behavioral videos. In *Adv. Neural Inf. Process. Syst. 32* (NeurIPS, 2019).
137. Nassar, J., Linderman, S., Bugallo, M. & Park, I. Tree-structured recurrent switching linear dynamical systems for multi-scale modeling. *ICLR* https://openreview.net/forum?id=HkzRQhR9YX (2019).
138. Glaser, J. I., Whiteway, M., Cunningham, J. P., Paninski, L. & Linderman, S. W. Recurrent switching dynamical systems models for multiple interacting neural populations. In *Adv. Neural Inf. Proc. Syst. 33* (NeurIPS, 2020).
139. Zoltowski, D., Pillow, J. & Linderman, S. A general recurrent state space framework for modeling neural dynamics during decision-making. *PMLR* **119**, 11680–11691 (2020).
140. Sussillo, D. & Barak, O. Opening the black box: low-dimensional dynamics in high-dimensional recurrent neural networks. *Neural Comput.* **25**, 626–649 (2013).
141. Vyas, S., Golub, M. D., Sussillo, D. & Shenoy, K. V. Computation through neural population dynamics. *Annu. Rev. Neurosci.* **43**, 249–275 (2020).
142. Ben-Yishai, R., Bar-Or, R. L. & Sompolinsky, H. Theory of orientation tuning in visual cortex. *Proc. Natl Acad. Sci. USA* **92**, 3844–3848 (1995).
143. Skaggs, W. E., Knierim, J. J., Kudrimoti, H. S. & McNaughton, B. L. A model of the neural basis of the rat's sense of direction. *Adv. Neural Inf. Process. Syst.* **7**, 173–180 (1995).
144. Kim, S. S., Rouault, H., Druckmann, S. & Jayaraman, V. Ring attractor dynamics in the *Drosophila* central brain. *Science* **356**, 849–853 (2017).
145. Yamins, D. L. K. & DiCarlo, J. J. Using goal-driven deep learning models to understand sensory cortex. *Nat. Neurosci.* **19**, 356–365 (2016).
146. Nayebi, A. et al. Task-driven convolutional recurrent models of the visual system. *Adv. Neural Inf. Proc. Syst.* **31**, 5290–5301 (2018).
147. Sussillo, D., Churchland, M. M., Kaufman, M. T. & Shenoy, K. V. A neural network that finds a naturalistic solution for the production of muscle activity. *Nat. Neurosci.* **18**, 1025–1033 (2015).
148. Kriegeskorte, N., Mur, M. & Bandettini, P. Representational similarity analysis — connecting the branches of systems neuroscience. *Front. Syst. Neurosci.* **2**, 4 (2008).
149. Wadhams, G. H. & Armitage, J. P. Making sense of it all: bacterial chemotaxis. *Nat. Rev. Mol. Cell Biol.* **5**, 1024–1037 (2004).
150. Frankel, N. W. et al. Adaptability of non-genetic diversity in bacterial chemotaxis. *eLife* **3**, e03526 (2014).
151. Waite, A. J. et al. Non-genetic diversity modulates population performance. *Mol. Syst Biol.* **12**, 895 (2016).
152. Vogels, T. P., Rajan, K. & Abbott, L. F. Neural network dynamics. *Annu. Rev. Neurosci.* **28**, 357–376 (2005).
153. Chen, X., Randi, F., Leifer, A. M. & Bialek, W. Searching for collective behavior in a small brain. *Phys. Rev. E* **99**, 052418 (2019).
154. Williams, A. H., Degleris, A., Wang, Y. & Linderman, S. W. Point process models for sequence detection in high-dimensional neural spike trains. *Adv. Neural Inf. Proc. Syst.* **33**, 14350–14361 (NeurIPS, 2020).

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