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# Whole-brain interactions underlying zebrafish behavior

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Detailed quantification of neural dynamics across the entire brain will be the key to genuinely understanding perception and behavior. With the recent developments in microscopy and biosensor engineering, the zebrafish has made a grand entrance in neuroscience as its small size and optical transparency enable imaging access to its entire brain at cellular and even subcellular resolution. However, until recently many neurobiological insights were largely correlational or provided little mechanistic insight into the brain-wide population dynamics generated by diverse types of neurons. Now with increasingly sophisticated behavioral, imaging, and causal intervention paradigms, zebrafish are revealing how entire vertebrate brains function. Here we review recent research that fulfills promises made by the early wave of technical advances. These studies reveal new features of brain-wide neural processing and the importance of integrative investigation and computational modelling. Moreover, we outline the future tools necessary for solving broader brain-scale circuit problems.

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## Introduction

While the vertebrate brain is large, containing many functionally distinct regions, diverse cell types, and billions of synapses, until recently neuroscience was restricted to studying only small fractions of the brain at a time. Advances in imaging of neural activity [1] and microelectronics [2] now provide activity data from thousands of neurons and suggest that brain function is powered by activity distributed over large numbers of spatially dispersed neurons [3] that encode various information related to perception and behavior. However, these methods still fall short of delivering a complete

dynamic view of the brain, as studies in mammals struggle to locate, define, and manipulate cell types or compare distant brain regions. This is why the translucent zebrafish *Danio rerio* has steadily gained traction for neuroscience [4]. For similar reasons, the fruit fly *Drosophila melanogaster* [5] and the nematode *Caenorhabditis elegans* [6] represent excellent systems to study brain-scale dynamics, but the zebrafish remains the only vertebrate permitting brain wide experimental access during behavior. Fast volumetric microscopy techniques [7,8] enable observations of dynamic neural processes across the entire zebrafish brain at micrometer resolution, impossible in other vertebrates [9]. Concurrently, new light-based biosensors have evolved rapidly, allowing for control and visualization of neural activity using high-performance calcium [10], GABA [11], dopamine [12], and bright, stable voltage sensors [13]. By harnessing these optical advances in combination with detailed characterization of behavior [14], perturbations [15], and modelling [16], the field is beginning to dissect information processing in the zebrafish's manageable population of ~100 000 neurons. After reviewing neurobiological advances in zebrafish, we will also discuss whether inspecting the neural activity of (almost) all neurons is merely convenient for homing in on relevant microcircuitry or whether there are fundamental principles to be learned by querying the entire brain.

## The value of a holistic approach to neural recordings

First, what really counts as 'whole-brain' recording? Recently, phosphorylated extracellular signal-regulated kinase (pERK) [17] has been used to report snapshot of activity across the entire zebrafish brain, yet the neural dynamics are completely lost. On the other hand, traditional two-photon laser scanning microscopy typically record fast neural calcium dynamics on single optical planes, so whole-brain recordings must be assembled computationally [18,19] from sequential acquisitions that are often minutes or hours apart. In other words, while such approaches *span* the entire brain, they do not capture simultaneous whole-brain activity (with some notable exceptions [20]). Alternatively, volumetric light-sheet microscopy [21] permits imaging at multiple brain volumes per second, and light-field microscopy offers outstanding temporal resolution but is currently limited in spatial resolution [22]. Yet, assuming real-time whole-brain imaging will be routine in zebrafish, what will be gained beyond reaching the abstract goal of completeness? Most brain-wide imaging studies emphasize its utility for agnostic searches for task-activated brain areas (Table 1), although simultaneous whole-brain access is

Table 1

## Overview of recent studies investigating whole brain interactions

Behavior/phenomenon	Focus Brain areas	Imaging Method	Causal Manipul.	Cell specificity	Model	Remarks	Ref.
Nociception					***	Convolutional Neural Networks	Haesemeyer et al., [16]
		●			***	Heat navigation	Haesemeyer et al., [64]
	Ht	●	⊗ △	*		pERK	Wee et al., [17]
	Ht	●		**		MultiMAP	Lovett-Barron et al., [46]
Auditory & Vestibular		■			*	Rotating light-sheet	Migault et al., [8]
		▲			*	Optical otolith trapping	Favre-Bulle et al., [37]
		●			*	Sound processing	Privat et al., [39]
Visuomotor Behaviors	Hb	■	⊗ △ ♀	*	**	Glia cells	Mu et al., [23]
	Th	▲	⊗		*	Looming detection	Heap et al., [30]
		▲	♀		*	Graph theory	Vanwalleghem et al., [38]
	Cb	● ▲		***	*	Electrophysiology	Knogler et al., [42]
		■			*	Various visual stim.	Chen et al., [4]
	Ot, Pt	●			**	Receptive field mapping	Wang et al., [27]
		●			*	Vis. stimulus design	Kist & Portugues [28]
		●			***	Higher order motion	Yildizoglu et al., [29]
	Pt	●			*	FuGIMA, Atlas	Kramer et al., [32]
	Hb	●			***	Evidence Accum.	Bahl & Engert [18]
IPN	●			***	Evidence Accum.	Dragomir et al., [33]	
Hunting Behaviors/ Appetite	Ot, Pt	●	⊗ △	**	*	Electroporation	Antinucci et al., [34]
	Ht	○	△			pERK	Wee & Song et al., [41]
	Ha, DRN	●	△ △	**	***	Light-Field Imaging	Andalman et al., [22]
	DRN	■			*	Tracking microscope	Marques et al., [24]
		●	⊗		*	Morphology	Henriques et al., [35]
Sleep		■			*	Polysomnography	Leung et al., [43]
		-			*	Galanin	Reichert et al., [44]
		-	⊗ △		*	Serotonin	Oikonomou et al., [45]
Miscellaneous	Cb	■			**	Operant conditioning	Lin et al., [25]
		●	△			Holographic photostim.	dal Maschio et al., [67]
	Sc	■	⊗	**		Cell tracking	Wan et al., [7]
		○		**		pERK, gene screen	Thyme et al., [52]
		○		**		Cellular atlas	Kunst et al., [31]
	Pt	■		**		Voltage imaging	Abdelfattah & Kawashima et al., [13]
	■	⊗			Online algorithms	Vladimirov et al., [56]	

Many recent studies aiming to reveal neural mechanisms underlying behavior or perception in zebrafish used cellular resolution functional *in vivo* imaging approaches such as light-sheet (■), selective plane illumination microscopy (▲), two-photon laser scanning microscopy (●), or confocal microscopy (○). Non-live imaging (-) to reveal neural activity using phosphorylated extracellular signal-regulated kinase (pERK), anatomical or molecular features have been used as powerful complementary tools. Most studies, after a brain-wide activity screen, focused on select brain regions to elucidate specific circuit mechanisms in one or more brain area: Ht, hypothalamus; Hb, hindbrain; Th, thalamus; Cb, cerebellum; Pt, Pretectum; Ot, Optic tectum; IPN, interpeduncular nucleus; Ha, habenula; DRN, dorsal raphe nucleus; Sc, spinal cord. To establish causal relationships various specific circuit perturbations were used: ⊗ laser ablations, △ photoactivation, ▽ photoinhibition, ♀ pharmacological approaches. Increasingly, studies combine functional profiling with cell type specificity (\*), using specific neurotransmitter or Gal4driver lines (\*\*), and even genetically unique cell types or tracing of single cells (\*\*\*). Finally, analysis and modeling approaches in these investigations vary in their level of complexity from regression mapping and qualitative circuit models (\*), highly quantitative descriptions (\*\*), to quantitative circuit models (\*\*\*).

not strictly necessary. While these searches are often used to generate correlational maps, the field is developing new analyses that reveal more about local circuit mechanisms [23\*\*], and there is a growing appreciation for more holistic views of network-wide activity patterns related to brain state transitions [22\*\*,24\*\*,25\*\*]. Beyond completeness, one of the major advantages of simultaneous, whole-brain analysis is that it may help unravel mysteries of redundancy and state-dependent encoding that are ubiquitous across species [3–6,24\*\*]. These redundancies may act as alternative pathways, broadcasters for motor commands downstream of sensory processing, error signals, or other state-dependent information necessary for parallel, multi-scale behavioral coordination, and zebrafish are uniquely positioned to adjudicate between these roles. Yet, while we report here that comprehensive sampling to generate correlation maps have become standard and insights into the encoding of brain states start to emerge, addressing these redundancies remains largely unexplored. Below we review recent progress using various forms of whole-brain analysis to discover both detailed microcircuits and insights about brain network functions underlying a range of behaviors, placing zebrafish at the center of efforts to solve models of whole-brain collaboration.

### **Agnostic search strategies to identify behaviorally relevant brain regions**

With the advent of large-scale imaging of neural activity, it is now possible to conduct a broad search of the entire zebrafish brain to conduct an unbiased sample of the regions that might contribute to the behavior in question. Such agnostic search strategies allow researchers to determine the neuronal circuitry underlying behavior (Figure 1a). Many early studies have leveraged the zebrafish visual system, which is highly conserved [26] and well developed at day five post fertilization, to generate models and characterize circuit components [27], using common strategies involving behaviorally relevant visual stimuli during brain-wide imaging. Exemplifying this strategy, two recent studies paired careful stimulus design with regression analysis to reveal the complex information processing across the brain supporting how local, light-dark transitions [28] and naturalistic higher-order motion detection induce optomotor responses [29]. Using reverse correlations to agnostically search for specific neural activity, these studies deconstructed visual circuits centered around the central retinal targets: the optic tectum, pretectum and parallel thalamic pathways [30]. Still others have used correlational mapping to reveal how various visual stimuli drive convergent activity in neurons in the anterior hindbrain [4], producing intricate topologies of sensory and motor brain areas. Following these functional characterizations, many studies use local circuit analysis to refine connectivity hypotheses after identifying relevant brain areas. For instance, Kramer *et al.* used precise photoactivation of functionally

characterized motion-sensitive pretectal neurons in conjunction with a recently developed single-cell brain atlas [31\*] to quantify morphological differences and projection patterns [32]. Finally, theoretical modelling approaches are often used to distill neural activity patterns, refine brain-scale mapping, and subsequent local circuit analysis. For instance, publications by Bahl *et al.* [18\*] and Dragomir *et al.* [33\*] demonstrate how distributed neural activity driven by classic random dot motion paradigms can be further screened for neurons whose complex temporal dynamics match-specific theoretical aspects of evidence accumulation, such as leaky integrator time constants.

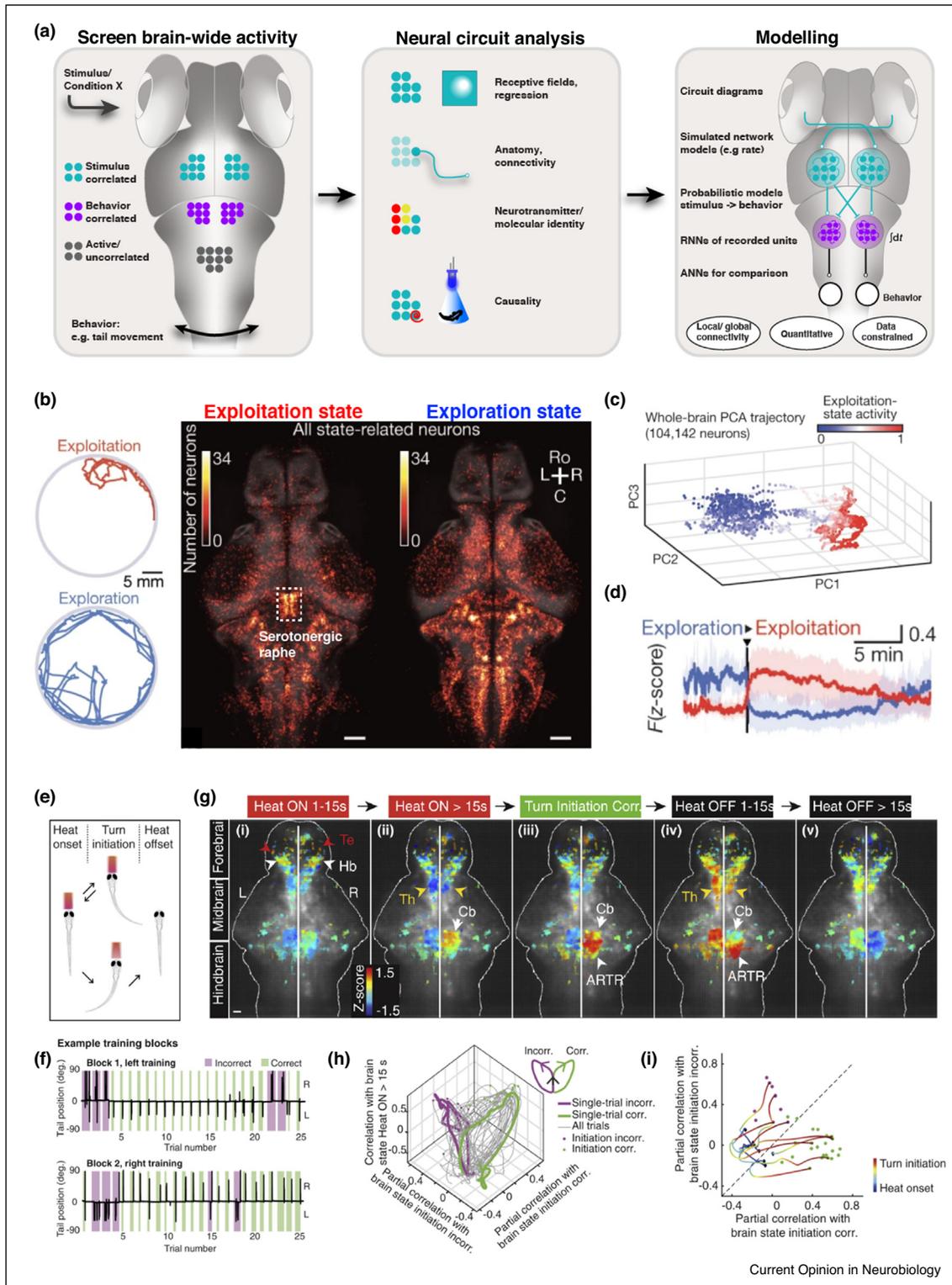
Whole-brain search has also been used to study zebrafish prey capture behavior, which involves more complex sensorimotor processing than the visual reflexes above. Bianco *et al.* used whole-brain imaging during a virtual prey capture assay to reveal specific retinorecipient neurons serving as command neurons for hunting initiation [34]. This study is a showcase for how the agnostic, systematic search for neural correlates leads to identification and characterization of genetically targetable neurons that link sensory processing to specific motor execution. Follow-up studies show that neural activity in distant cholinergic neurons of the *nucleus isthmi* [35] appear to sustain binocular, intertectal processing during hunting behaviors [36].

Vestibular processing poses interesting challenges for imaging, but recent use of miniaturized [8] or tilting light-sheet microscopy has permitted brain-scale imaging during physiological or fictive body rotations [37]. These experiments reveal vestibular processing to be a widespread bilateral phenomenon, with multiple, distributed, functionally distinct clusters. Additionally, presenting water flow with microfluidic devices [38] and auditory stimuli [39] revealed diverse flow and sound responsive neurons distributed across the brain. Ultimately, it will be interesting to learn how the zebrafish brain integrates information across the different modalities activated during behavior.

### **Towards integrative views on brain state transitions**

Increasingly, investigations now extend zebrafish brain mapping by focusing on brain state transitions [40]. Recently, innovative tracking microscopy for monitoring brain-wide neural activity while zebrafish hunt for live prey [24\*\*] revealed opposing oscillatory neural network states during foraging (Figure 1b–d). These network states correspond to minutes-long exploratory and exploitative phases in which fish either increase overall locomotion or engage in prey capture behaviors, respectively. Using linear regression to identify neurons with activity profiles correlated with various movement types, eye position, and prey detection and ingestion, Marques

Figure 1



Brain states encoded in network activity and local circuitry.

(a) The common strategy to use whole-brain regression analysis under various conditions and behavioral recordings often reveals widespread sensory (teal) and motor (purple) related signals. After task relevant neurons are located, most studies progress with local circuit analysis to refine response types, connectivity and anatomy, using molecular identity, and through the use of a combination of single cell electroporation, transgenic lines, and causal perturbations, including laser ablations of single neurons or optogenetic activation in concert with behavioral testing. These results are often synthesized into models that range from simple activity maps, suggesting circuit diagrams, to realistic quantitative network

*et al.* show that these internal states are encoded in brain-wide networks. Furthermore, they demonstrate that the anticorrelated, widespread neural activity patterns associated with these two sets of internal states is controlled by specific serotonergic neurons coupled with the required sensorimotor processing associated with prey capture. These findings suggest that these serotonergic neurons of the dorsal raphe nucleus (DRN) act as brain state ‘switching’ signal broadcasted to the rest of the brain. By recording from the entire brain, they can show that specific clusters of neurons in specific brain regions become activated in response to this broadcast signal, but that other neurons in other brain regions are suppressed by the same signal. This study exemplifies the importance of investigating brain-wide neural activity while monitoring behavior, as the observed neural dynamics related to both stimulus processing and internal brain state changes were understood only in the context of nuanced behavior over time.

Emphasizing the careful analysis of behavior, more static methods (pERK) have recently localized a different set of bidirectional, anticorrelated networks of neurons in the caudal and lateral periventricular zone of the hypothalamus associated with longer term hunger and feeding states [41]. Another study (Figure 1e–i) discovered the global recurring dynamics preceding a decision by combining fast volumetric light-sheet imaging during a novel operant conditioning task [25\*\*] in which fish have to flick their tail in the ‘correct’ direction to ‘switch off’ a painful heat stimulus. The authors discovered that the neural dynamics can predict both the time of decision as well as the decision (motor output) itself on a trial-by-trial basis. These dynamics occur in a distributed interhemispheric

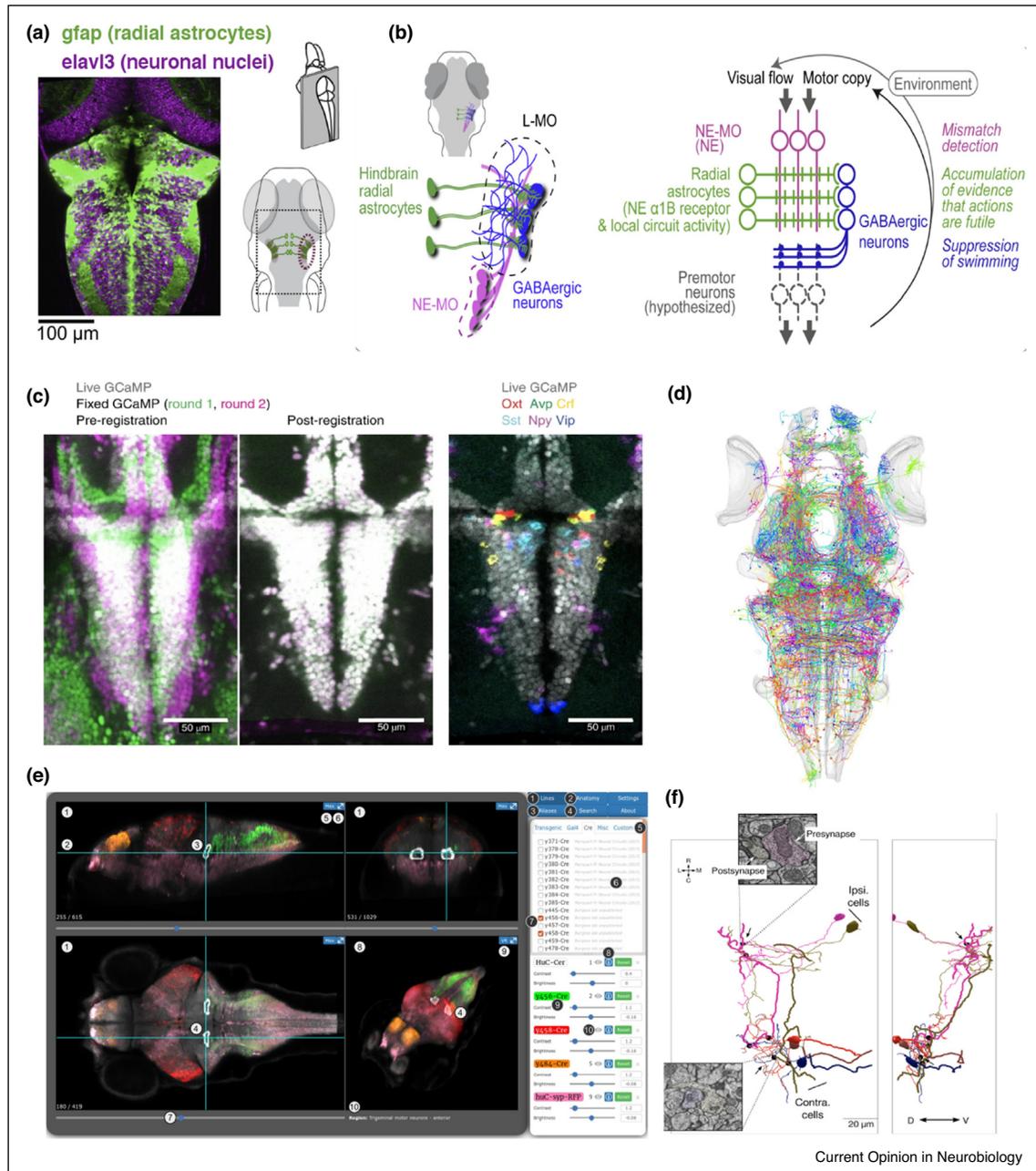
network, primarily between habenula and cerebellum. The study also highlighted a more cognitive role for the cerebellum, with predictive pre-motor activity more than 10 s before movement initiation being largely localized to granule cells for individual trials. Finally, the authors propose a circuit model in which the ‘timing of movement’ is predicted by ramping bilateral cerebellum neural activity and the ‘tail flick direction’ is computed by ipsi and contralateral cerebellar competition. Together with the recent characterization of visually evoked spatiotemporal dynamics across the entire Purkinje cell population [42], these investigations further underscore the importance of analyzing neural activity in the context of behavior.

Recent holistic neural recordings of sleep deprived zebrafish revealed the neural signatures of sleep, which had been elusive in species lacking traditional cortical structures. Leung *et al.*, using non-invasive polysomnography, show conservation of slow-wave and rapid eye movement sleep, as well as a role for the conserved melanin-concentrating hormone, suggesting that common neural signatures of sleep emerged over 450 million years ago [35,43\*]. Further, brain-wide pERK mapping identified the neuropeptide Galanin in the preoptic hypothalamus as controlling rebound sleep, a state caused by sleep deprivation [44]. While the serotonergic neurons in the raphe appear to be direct modulators of sleep levels in both zebrafish and mice [45], this research suggests that sleep might be a brain-scale, if not body-scale, neurobiological phenomenon.

Furthermore, recent brain-scale recordings provide insights of how zebrafish process aversive information. By utilizing light-field microscopy during shock-induced

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- (Figure 1 Legend Continued)** simulations using recorded activity dynamics in recurrent neural network replicating biological effective connectivity. Other approaches include probabilistic models linking sensory input to behavioral output and comparison to artificial neural networks.
- (b)** In a recent study [24\*\*], focus shifted towards dynamic brain state transitions rather than correlational maps. Left, example behavior trajectories of zebrafish in exploitation (red) and exploration (blue) states. Right, locations of all neurons encoding these brain states, projected across 17 registered fish recorded with a tracking microscope, revealing brain state switches triggered by serotonergic neurons (dashed box). Ro, rostral; C, caudal; L, left; R, right.
- (c)** PCA trajectory of whole-brain activity (104,142 neurons) from a representative animal, color-coded by the activity of exploitation-state-encoding neurons.
- (d)** Average activity (mean  $\pm$  s.d.) of exploitation-state-encoding (red) and exploration-state-encoding (blue) neurons across the transition from exploration to exploitation (top). These dynamics are likely key to understand brain wide states that modulating other processes.
- (e)** Lin *et al.* [25\*\*] used an operant conditioning assay combined with whole-brain calcium imaging to investigate how decision brain states develop over time. Head-fixed larval zebrafish receive a mildly aversive heat stimulus by an infrared laser (red trapezoid) at the beginning of a trial. The laser is turned off if the fish makes a tail movement in the reward direction and remains on otherwise. In the second training block the reward direction is switched, with each block consisting of 20–25 trials.
- (f)** The learning progress of an example learner fish. Black traces indicate tail positions over time, magenta and green rectangles indicate the duration of the heat stimulus for each incorrect and correct trial, respectively.
- (g)** Temporal evolution of brain states reveals distributed brain activity and bihemispheric preparatory activity. ARTR, anterior rhombencephalic turning region; Cb, cerebellum; HB, habenula; Te, telencephalon.
- (h)** Performance-dependent bifurcation of brain states before turn initiation. Left, after heat onset, brain states exhibit similarity along the ‘Heat ON’ dimension (vertical axis), followed by a pre-turn bifurcation toward the correct or incorrect state. Similarity is measured by partial correlation between a given brain state and the average correct, incorrect, or ‘Heat ON’ state. Right, representation of single-trial bifurcation process. Individual correct and incorrect trials are highlighted during the pre-motor period (black diamonds: heat onset, green and magenta dots: turn initiations for correct and incorrect turns, respectively).
- (i)** 2D representation of single-trial bifurcation process. Individual correct and incorrect trials are highlighted during the pre-motor period (black diamonds: heat onset, green and magenta dots: turn initiations for correct and incorrect turns, respectively).

Figure 2



The importance of diverse cell types across the brain.

**(a)** Mu *et al.* [23<sup>\*\*</sup>] studied brain wide responses of radial astrocytes (green) and neurons (magenta) while either providing or withholding visual feedback. Dual color whole-brain neuronal and astrocyte  $Ca^{2+}$  imaging (GCaMP6f and jRGECO1b), concurrent with fictive behavior monitoring and visual stimulus delivery, showed dynamic increases in specific cell populations during epochs of futility-induced passivity. While neuronal brain-wide activity was higher during active behavior than passivity, brain-wide glial  $Ca^{2+}$  signals increased before passivity onset and remained elevated during passivity.

**(b)** In an experimental tour de force combining optogenetics, specific ablations and pharmacology, radial astrocytes are demonstrated to be necessary and sufficient for induction of passivity. Mismatch signals from norepinephrine neurons are integrated over time and using an unknown signaling mechanism behaviors are shut down through local GABAergic inhibition.

**(c)** Barron-Lovett *et al.* [46<sup>\*</sup>] investigated how fish respond to aversive stimuli, such as high salinity. By using their potent MultiMAP [75] approach, live neural activity volumes are registered with post-fixation and staining volumes. Here, this approach aligns neuropeptidergic identities with functional properties in the preoptic hypothalamus.

**(d)** Schematic illustrating the remarkable searchable online repository of over 2000 single neurons registered and aligned by Kunst *et al.* [31<sup>\*</sup>]. This represents a strategy to leverage the single-neuron atlas (also used by Kramer *et al.*) to determine long range projections that are difficult to trace with current photoactivatable fluorescent proteins.

passive coping behaviors, Andalman *et al.* revealed that transitions to coping behaviors are orchestrated by an increased recruitment of habenular neurons [22\*\*]. Wee *et al.* used pERK mapping to identify brain regions processing noxious information, revealing convergent nociceptive pathways in zebrafish. Localized to the hypothalamic preoptic area, oxytocin neurons reflect broad classes of noxious stimuli and their activation is sufficient to trigger defensive behavior recruiting brain stem premotor regions [17]. Similarly, whole-brain analysis uncovered diverse peptidergic neurons in the hypothalamus driving defensive behaviors to homeostatic threats, such as high salinity [46\*]. These studies are important steps towards integrative understanding of how specific cell types with distinct molecular expression profiles collaborate to regulate brain state transitions.

### Experimental access to defined cell types across the brain

The previous sections described how whole-brain imaging can be used to delineate networks and circuits based on their functional properties. But detailed circuit dissection benefits from genetic methods that target-specific cells in the brain. Intersectional genetic methods, e.g. Gal4 driver lines, allow restriction of sensor or actuator expression in only neurons that co-express multiple drivers, granting unique access to genetically defined neural populations [47]. Comprehensive single cell RNA-Seq maps can also be used to develop cell-specific genetic access [48]. Illustrating the power of cell-specific access in the whole-brain zebrafish paradigm, Mu *et al.* used an agnostic search strategy by which they simultaneously imaged both glial and neuronal activity (Figure 2a), examining regions involved in futility-induced passivity behavior [23\*\*]. Specifically, they presented visual motion stimuli in either closed-loop or open-loop configuration. The latter configuration, in which the visual stimulus speed is not modulated by the behavior, caused the fish to enter a phase of ‘giving up’, ceasing all swim activity. They demonstrate that these glia cells called radial astrocytes serve as key evidence accumulators before zebrafish ‘give up’ swimming when visual feedback is decoupled from behavior. Suggesting an unexpected computational role, these radial astrocytes integrate signals encoding the lack of visual feedback and are both necessary and sufficient to drive this ‘induced passivity’ behavior through a GABA-mediated mechanism (Figure 2b). This study exemplifies the power of brain-wide imaging followed

by causal manipulations in defined cell types. The authors used targeted optogenetic photoactivation and photoinhibition, specific laser ablations, and pharmacology, showcasing how whole-brain imaging with cell type specificity yields novel insights into global and local circuit function. An alternative strategy for establishing such cell-specific associations is to register brain-scale activity maps with multiplexed gene expression profiling (Figure 2c). Furthermore, the field is prioritizing online brain atlas repositories [31\*,47,49,50] to dynamically compare, assess and evaluate incoming findings of anatomy, expression profile, and activity (Figure 2d–e). The remarkable acceleration of sequencing and molecular techniques have also enabled reconstruction of the entire developmental trajectory of every single cell in the zebrafish [51] and evaluation of neuropsychiatric disease related risk genes [52] using CRISPR/Cas9 [53]. Together with electron microscopy reconstructions revealing connectivity [54,55], the ability to target-specific molecularly defined cell types represent important tools to dissect the logic of neural circuits underlying behavior.

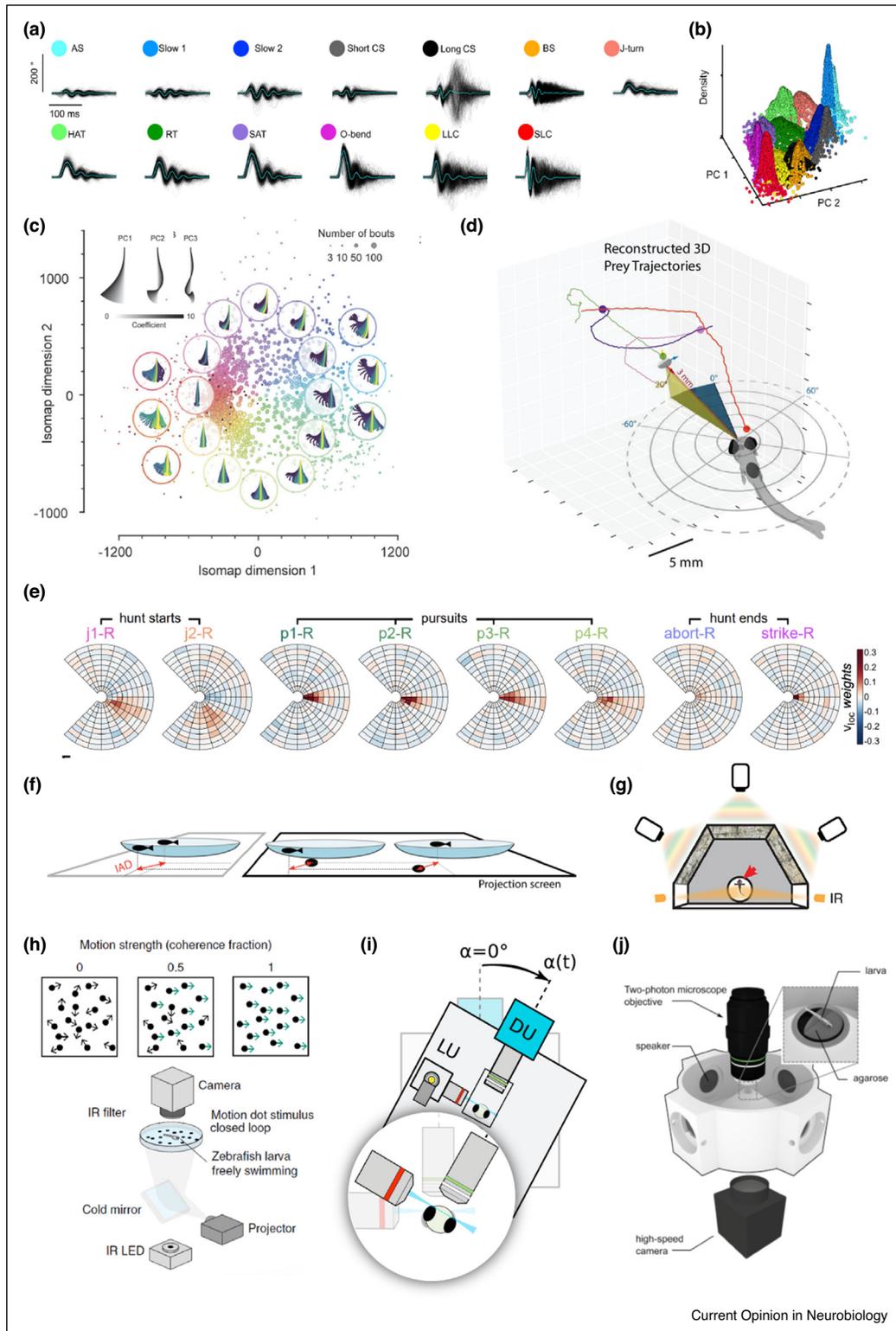
### Towards comprehensive behavior quantification underlying whole-brain dynamics

Ultimately, the brain serves to control behavior, and one of the strengths of the zebrafish is the relative ease to quantify their behavior. Ultimately, unscrambling complicated whole-brain networks will require intricate quantitative descriptions of circuit computations transforming sensory input into behavior. To gain precise control of the sensory environment, various virtual reality paradigms are being used [56,57], e.g. to model responses to light gradients [58]. Importantly, high-quality, open-source methods for tracking [59,60] and unsupervised clustering (Figure 3a–e) of swim kinematics [14] are setting standards for complex behavioral analysis. Comprehensive, large-scale recordings of thousands of swim events allowed Mearns *et al.* [61] and Johnsen *et al.* [62] to visualize entire kinematic ethograms and to generate probabilistic models of behavior, revealing the structure of behavioral state transitions on short and long time-scales. Both studies highlight the importance of past actions and sensory experience, linking behaviors in statistically predictable ways to generate hypotheses for underlying neural dynamics. In another study, using careful behavioral analysis to generate testable

(e) Graphical user interface of *Zbrowser* [47] with multiview options of Cre and Gal4 transgenic lines. Similar atlases such as the *Z-brain*, maintained by Engert *et al.*, and important cellular atlases [31\*] are invaluable tools elevating zebrafish scientific community by enabling fast comparisons of task relevant brain regions and visual exploration of expression profiles.

(f) Serial electron microscopy to reconstruct neurons identified via functional calcium imaging [55], will allow to determine circuit-specific connectivity. Here, three views of synaptically connected integrator neurons encoding eye positions. Synapses from the ipsi-only group onto another neuron from the ipsi-only group (top left) (black circles) and from the ipsi-only group onto contra-only neurons (right). Black arrows indicate the location of the synapses, with insets showing the electron micrographs at two representative locations; colors in the insets are representative of the cells to which the synapses belong.

Figure 3



Large scale behavioral analysis brings the entire ethogram into view.

(a) Zebrafish swim in discrete swim bouts. These elementary movements can be clustered into 13 distinct swim types [14], as shown here as angle of tail segment (°) versus time (ms), with one hundred randomly picked bouts of each type (black). Cyan lines are the average of all bouts in each category. Colored dots next to bout abbreviation correspond to (b). Such careful assessment of behavior is critical to understand how the brain transforms any signals into distinct types of behavioral outputs. These 13 distinct swim types include: approach swims (AS), two distinct

predictions of dynamic computation, 3D tracking and modeling of hunting zebrafish and their live prey suggest that zebrafish brain utilize implicit predictive models of the environment [63]. With the technical progress to record from large populations of neurons in freely swimming fish connecting quantitative models of behavior and investigating how internal state variables guiding naturalistic behavior emerge over time appears within reach [24\*\*]. Recently, Haesemeyer *et al.* showed that artificial neural networks (ANN) navigating heat gradients can be a powerful tool for understanding neural dynamics [16], where they reported emergence of artificial neurons encoding stimulus derivative over time in the same way as the zebrafish brain [64]. Furthermore, recurrent neural networks fit to neural responses have been used to predict and explain effective connectivity changes within and between brain regions during behavioral state changes [22\*\*]. As in other animal models [65], convergent ANNs should enable modeling studies of experimentally inaccessible systems. Finally, we note the value of comprehensive neural recordings to investigate how cascading spontaneous activity propagates throughout the entire brain volume, phenomena termed ‘neuronal avalanches’ [66]. Such studies inform models predicting how the entire system responds to external or internal state changes.

### Future breakthroughs

Overall, we predict that while high-quality, fast functional imaging will become easier, we must continue to develop other necessary tools. There is a critical need to streamline and integrate data collection, storage, processing, annotation, analysis, visualization, and interpretation. Additionally, the field requires user-friendly computational tools for online imaging analysis that would permit interactive experimental design [56], e.g. by acting

on information generated during the experiment. Such methods for adaptive experimental design will be necessary for comprehensive functional connectivity mapping using precise optogenetic manipulation [67]. Routine electron microscopy reconstructions [54,55] and effective transsynaptic tracing methods, like cell type-specific viruses [68], will be crucial for confirming hypothesized connectivity. Fortunately, efforts to combine functional imaging and complete synaptic EM reconstructions are underway [69]. Extensions of imaging paradigms to older fish [57], as well as other fish species potentially more suitable for studying complex behaviors and brain function [70,71], may be crucial to model circuits not yet developed in the larval zebrafish. These complex behaviors include important new work on shoaling, which opens up research into how socially relevant stimuli are processed in the brain [72], possibly under genetic control [73]. Ultimately, given the size and complexity of whole-brain zebrafish datasets, the field may benefit most proximally from a common interactive framework to share and search multiscale information, from molecular to morphological to functional neural activity data. Initiatives such as *neurodata.io*, which supports large-scale storage of anatomical and activity data on the cloud, will serve as strong foundations for collaboration and provide useful datasets for developing new analytic tools and machine learning algorithms [74].

### Conclusions

The zebrafish is still relatively young as a systems neuroscience model but has practical advantages that have already generated new insights into brain-wide sensorimotor transformations. Investigating brain-scale neural dynamics in zebrafish has become a routine tool for uncovering regional microcircuit level computations and also for discovering how networks collaborate

**(Figure 3 Legend Continued)** slow swims (Slow 1, Slow 2), capture swims (Short CS, Long CS), burst swims (BS), large turns for prey capture (J-turns), high angle turns (HAT), routine turns (RT), spot avoidance turns (SAT), abrupt high angle turns (O-bend), and two remaining escape responses (c-starts) of long-latency (LLC) or short-latency (SLC).

**(b)** Bout density versus the first two principal components of the movement space used to categorize all bouts. Colors represent bout categories from (a).

**(c)** Similar bout-type analysis [61] measures the kinematic difference between every pair of bouts with dynamic time warping (DTW) to conclude that behavior structure is largely continuous. Non-linear isomap embedding shows bouts in single behavioral space, with some local densities representing more stereotyped types of forward swimming. Inset: Dimensionality reduction of the first three ‘Eigenfish’.

**(d)** Bolton *et al.* [63] used a novel 3D assay for continuous tracking of eye angle, yaw, pitch, and tail angles mapping prey trajectories to fish movement choices. Cartoon shows a fish hunting live prey, paramecia. Prey features are mapped to a spherical coordinate system originating at the fish’s mouth. Angular prey position and velocity construct a future estimate of prey position to guide hunting behavior.

**(e)** Johnson *et al.* [62] used large scale behavioral tracking that allowed them to use probabilistic modeling to characterize the likelihood of visual stimuli preceding for each rightward hunting bout type.

**(f)** Larsch *et al.* [72] recently showed that motion cues induce social, shoaling-like behavior in juvenile fish (21 days post fertilization), though imaging of associated brain function remains under way. Schematic of a virtual social assay used in the study, whereby each animal sees black dots underneath at the location of the other three animal(s), virtually pairing them.

**(g)** Huang *et al.* [57] recently demonstrated how virtual reality rigs can be used to present visual stimulation to zebrafish in realistic 3-D environments, enabling adult virtual reality.

**(h)** Dragomir *et al.* [33\*] (and Bahl *et al.*) have recently employed classic random dot motion paradigms to flexibly investigate evidence integration. Zebrafish offer a strong behavioral model to investigate sensory integration tasks traditionally reserved for primate research.

**(i)** Schematic of the rotating light-sheet microscope demonstrated by Migault *et al.* [8], used to monitor the activity of the vestibular system. This microscope includes separate illumination (LU, light-sheet unit) and detection (DU, detection unit) pathways that rotate with the fish.

**(j)** Delivery of acoustic stimuli delivered via waterproof speakers [39] allows brain-wide imaging of auditory responses.

holistically. Achieving realistic, multiscale dynamic models will require fast imaging, precise perturbation technologies, sensitive biosensors and actuators delivered to specific cell types, advanced computational tools for acquisition, analysis, and annotation. Recent whole-brain studies integrate multiple levels of analysis spanning behavior, neuronal dynamics, genetic identity, and connectivity, and these studies establish new principles of interaction between neurons and brain regions across a wide range of behaviors.

## Conflict of interest statement

Nothing declare.

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