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Brain-wide, scale-wide physiology underlying behavioral flexibility in zebrafish

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The brain is tasked with choosing actions that maximize an animal's chances of survival and reproduction. These choices must be flexible and informed by the current state of the environment, the needs of the body, and the outcomes of past actions. This information is physiologically encoded and processed across different brain regions on a wide range of spatial scales, from molecules in single synapses to networks of brain areas. Uncovering these spatially distributed neural interactions underlying behavior requires investigations that span a similar range of spatial scales. Larval zebrafish, given their small size, transparency, and ease of genetic access, are a good model organism for such investigations, allowing the use of modern microscopy, molecular biology, and computational techniques. These approaches are yielding new insights into the mechanistic basis of behavioral states, which we review here and compare to related studies in mammalian species.

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Introduction: explaining complex behaviors requires brain-wide, scale-wide investigation

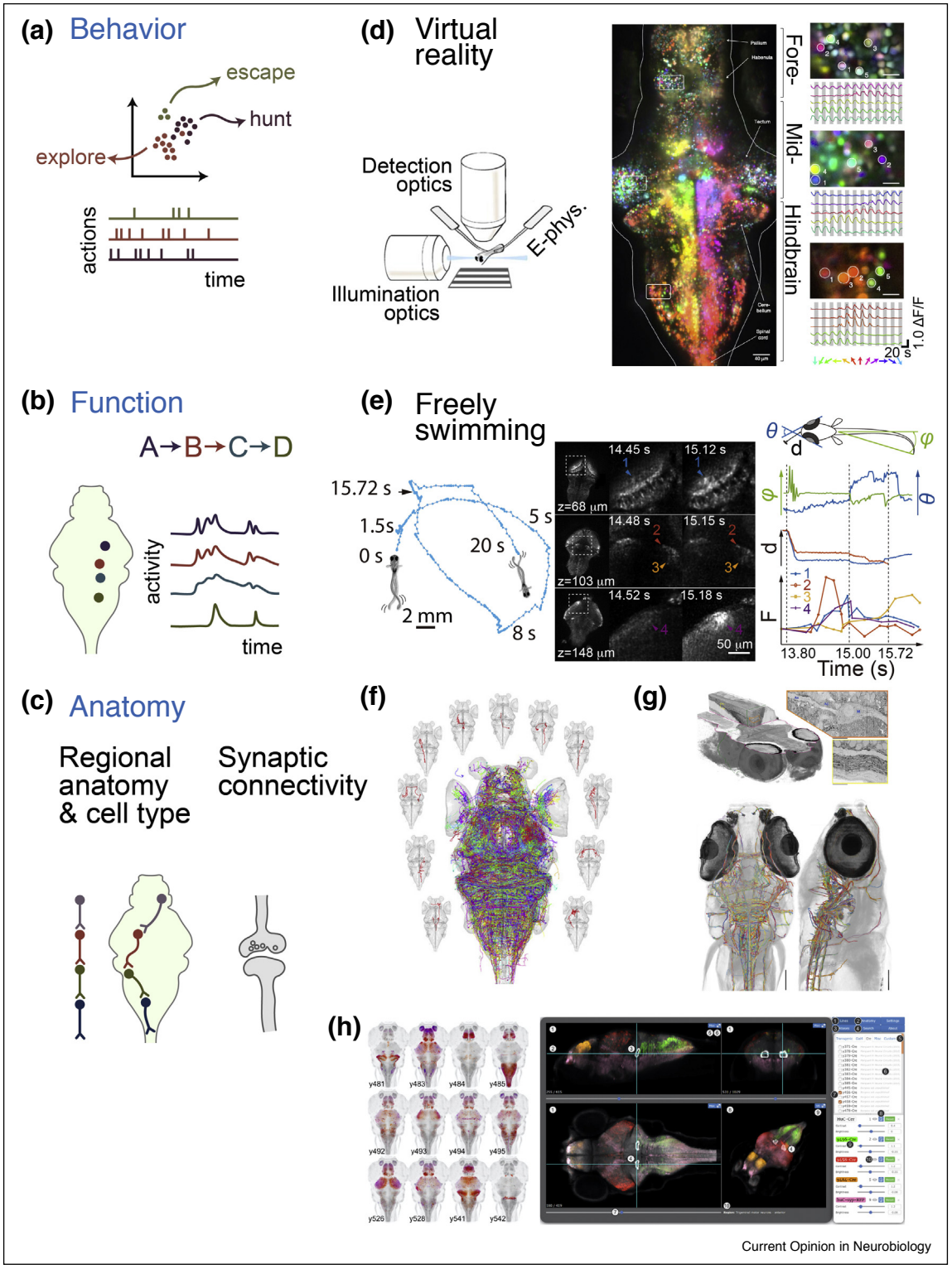
From single-celled organisms to mammals, behavioral flexibility allows animals to thrive amidst largely indifferent, sometimes hostile, environments. An animal's actions depend on the current conditions of its surroundings and its body, as well as on past experiences. For example, hearing a rustling sound, an animal may approach to investigate the potential presence of prey. But if the same animal had an encounter with a predator a few minutes earlier, it might instead avoid the rustling sound. When sufficiently hungry, however, the animal might approach regardless of the risk. Even behaviorally simple animals may have dozens of such algorithm-like components in their behavioral flexibility repertoire, whereas complex animals may have thousands. Some algorithmic components are common across phyla, while some are species-specific. The physiology that mediates behaviorally flexible processes is never localized to only one brain region. Instead, complex behavior tends to be generated through communication among large populations of neurons scattered across the brain that process various sensory, motor, and intermediate types of information. This general pattern holds across species, although the specifics of what information is extracted from the environment and what sets of motor patterns are triggered depend on the animal's individual and evolutionary history.

To address the distributed and multi-scale nature of neural computation, neuroscience has been moving toward creating techniques for observing and perturbing dynamics at multiple scales in behaving animals, exploiting synergies among microscopy, molecular biology, protein engineering, genetics, behavior tracking, and computational neuroscience (Figure 1a–c). On the molecular scale, genetically encoded or synthetic biosensors make it possible to track changes in the concentration of neurotransmitters [1] and neuromodulators [2,3], calcium levels [4], transmembrane voltage [5–8], and intracellular molecular pathways [9]. Microscopes with high spatial resolution can image such reporters at subcellular scale in living tissue [10]. On the macro scale, custom microscopes can image large volumes at high speeds and at cellular scale in behaving animals [11–17,18•] (Figure 1d,e) for functional analysis of cell activity [19,20]. There have also been rapid advances in the development of actuators that can induce or silence spiking or affect intracellular molecular pathways [21,22]. Breakthroughs in chemistry, molecular biology, and microscopy are allowing neuroscientists to extract more and more information about the large-scale and the small-scale dynamics of living tissue.

Linking zebrafish to other model systems

Animals with brains that are optically accessible, such as larval zebrafish, allow these methods to be used anywhere in the brain, *in vivo* and even during behavior, without

Figure 1



Whole-brain functional and structural analysis.
(a–c) Combining quantitative studies of behavior with recording of neural activity and anatomy across the whole brain is key to leveraging larval zebrafish for progress in systems neuroscience.
(d) Functional imaging and analysis in the fish brain, showing directional tuning to visual motion across the brain, and illustrating hypothetical models of information transfer between neurons. Whole-brain functional imaging of embedded larval zebrafish. Left, schematic of experimental

surgery. Furthermore, the small brain size and genetic accessibility of zebrafish have aided in the creation of important resources such as anatomical atlases [23,24,25•], a neuron morphology database [25•], and whole-brain anatomical ultrastructure [26•] (Figure 1f–h). Because zebrafish are vertebrates, there is much homology between their brains and the brains of mammals, and molecular function and neuromodulatory systems are largely conserved.

Animals base their actions not only on instantaneous observations of the world around them but also on temporally separated past experiences. Recent events in the environment or a current bodily condition may evoke a brain state that makes the animal more likely to take certain actions than others. For example, the presence of a conspecific competitor may make aggressive action more likely, but still contingent on what transpires next. Behavioral states that allow animals to navigate danger, hunger, social interactions, and other scenarios arise from brain computations operating on incoming sensory and bodily information, either abruptly or after gradual accumulation of information. Many behavioral states in zebrafish have clear analogies in other animal species, as discussed below, where we cover examples of insights from zebrafish on brain-state-dependent behavior and their proven or potential mammalian counterparts.

Targeted investigations of cross-species hypothalamic function

Drawing inspiration from work in mammals, several zebrafish studies have focused on well studied brain structures such as the cerebellum [27] and the hypothalamus. In mammals, the hypothalamus contains signals relating to hunger, social interactions, threat, and aggressivity, and can sustain related persistent behavioral states [28,29]. Related zebrafish studies revealed coding and connectivity principles of circuits for nociceptive behavior, hunger, habituation, and sleep [30–35]. Although the breadth of behavior in young zebrafish is more limited, the experimental advantages discussed above make it easier to resolve activity and circuit structure from large fractions of the brain, which can then feed back to inform future studies in rodents, primates, and other species.

Brain-wide investigations of cross-species serotonergic function

Another strategy is to start with a behavior of interest and use whole-brain imaging as an unbiased screen for neural activity related to that behavior, as far as imaging, sensors, and analysis approaches permit. One such study [36•] describes a simple but important persistent change in motor patterns that arises when animals need to change the vigor with which they move in response to changes in their environment or their body. This can happen, for instance, when water temperature drops, rendering muscles less effective and requiring stronger motor commands from the brain to make the fish swim with equal speed. In this study, whole-brain light-sheet imaging was used to survey brain-wide activity for neurons encoding the memory of learned motor vigor, leading to a focus on the dorsal raphe nucleus (DRN). A more detailed investigation of the DRN showed that visual feedback during swimming, indicative of how far the animal traveled as a result of a motor command, triggers activity in serotonergic neurons that encodes the distance traveled during a swim bout, which can be thought of as a representation of action efficacy (Figure 2b). Accumulating DRN activity caused a reduction of motor vigor, allowing the animal to flexibly tune motor vigor to the demands of changing environmental interactions. Whole-brain imaging during behavior was essential for finding the neural substrate for this vital computation in an unbiased manner, which strengthens the inferential power of subsequent phylogenetic comparisons by reducing confirmation bias.

The mammalian serotonergic system similarly reduces motor vigor [37] (Figure 2c). Furthermore, activating the raphe nucleus in behaviorally helpless rodents leads to renewed motor attempts [38], an indication that the raphe may be thought of as containing an internal representation of action efficacy, similar to the representation of swim efficacy in zebrafish. These parallels reinforce the value of studying related functions in both species, although the wider effects of the functionally and genetically heterogeneous populations of the dorsal raphe nucleus are still intensely under investigation [39].

A role of the DRN in persistent behavioral states was also observed in hunting behavior in larval zebrafish. Neurons

(Figure 1 Legend Continued) preparation; middle, maps of direction tuning derived from neuronal responses to visual motion; right, example cells from the habenula, tectum, and hindbrain. Black traces, averaged calcium responses; color traces, prediction of best-fitting tuning curve model. From Ref. [20].

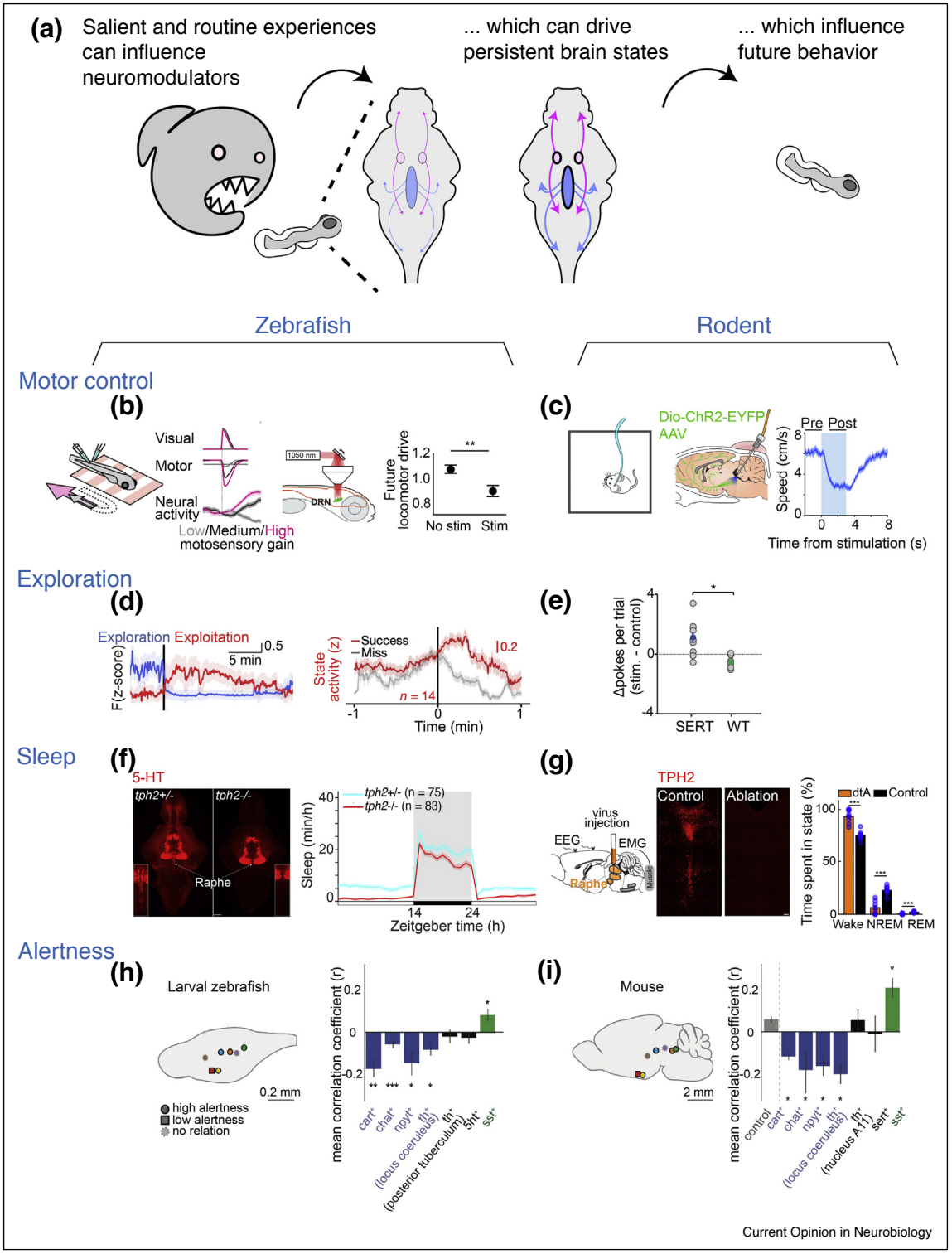
(e) Whole-brain functional imaging in freely swimming larval zebrafish during prey capture with confocal light-field microscopy. Left, trajectory of a freely swimming zebrafish during imaging; middle, snapshot of the brain during imaging; right, neural activity from example cells, aligned with prey capture behavior. Data from Ref. [18•].

(f) Digital atlas of larval zebrafish brain obtained from confocal imaging of over 2000 neuron morphologies. From Ref. [25•].

(g) Whole-brain serial-section electron microscopy in larval zebrafish. Top, example images; bottom, reconstruction from myelinated axons. From Ref. [26•].

(h) Zebrafish Brain Browser, a cellular resolution atlas with 264 transgenic lines registered to a standard brain. Left, Cre enhancer trap lines; right, web-browser based edition. From Ref. [23].

Figure 2



Neuromodulatory functions identified in zebrafish and rodents.
(a) Encoding past experiences and modulating future actions through neuromodulation.
(b) DRN encodes swim feedback and modulates motor vigor in zebrafish. Left, DRN activity changes according to effectiveness of motor actions: in zebrafish, DRN activity increased more when actions caused larger sensory feedback. Right, optogenetic activation of DRN decreases motor vigor. From Ref. [36•].
(c) In mice, optogenetic DRN stimulation can suppress motor vigor. From Ref. [37].

in this area modulate locomotion states between exploration and exploitation, as discovered with whole-brain imaging in freely swimming and hunting animals [40••]. Exploitation states, where animals hunt for prey locally, correlated with higher levels of raphe activity (Figure 2d, left). Notably, this state overlaps with the low-vigor state described above because, overall, less distance is traversed. Successful hunting triggered a small increase in DRN activity (Figure 2d, right), possibly related to activity increases seen during successful swimming in short-term motor learning, again suggesting a link between responses of neurons in the DRN and action efficacy [36•]. Downstream regions modulated by serotonin include sensory regions involved in hunting [33,40••]. In mammals, increased persistence in exploitation states also follows serotonergic raphe activation (Figure 2e) [41]. Thus, although the function of the serotonergic system is likely heterogeneous in different species and behavioral contexts and includes reward-related signals [42,43], consistent themes are emerging through multi-species studies aided by whole-brain functional screening in zebrafish.

Populations of neurons in the raphe also drive what is perhaps the most extreme behavioral state transition, wakefulness versus sleep, as found in a study of both zebrafish and mice, where results in one species directly inspired experiments in the other (Figure 2f,g) [44••]. In both species, DRN activity strongly modulated sleep. Ablation of the raphe reduced sleep, and tonic stimulation induced sleep, but burst stimulation induced wakefulness. By comprehensively studying genetics, pharmacology, activity measurements and perturbation, these authors reconciled previous seemingly contradictory results on the role of the serotonergic system in sleep. This study, along with other investigations into the neural basis of sleep in zebrafish, including interactions with non-neuronal cells [34,45], show how insights from different species can be leveraged to make fast and profound progress. Future investigations may address how genetically and functionally heterogeneous subpopulations of DRN neurons co-modulate these related but distinct serotonin-dependent behavioral states.

Combined influences of neuromodulators on behavioral states

Behavior can be modulated along more than one dimension by multiple neuromodulators simultaneously. This possibility was addressed in a combined study of zebrafish and mice (Figure 2h,i) [46••], where reaction times to predator-like stimuli were used to infer alertness: more alert animals react faster than less alert animals. Whole-brain calcium imaging was used to quantify how neuronal activity just before the stimulus correlated to alertness. To match function to cell type, after the experiment, the brains were processed for immunohistochemistry to identify the neuromodulator phenotype of each imaged neuron, and the functional data were registered to anatomical data at single-cell resolution. This work revealed many correlates, across multiple neuromodulatory systems, to alertness state. In the same study, several of the nuclei discovered in the zebrafish data were imaged in the mouse in an analogous alertness task, finding remarkable conservation across vertebrates of the correlations between activity in neuromodulatory centers and behavior.

In addition to the fluctuation over time of behavioral patterns within an individual, the frequency and potency of behavioral changes also varies across individuals and across generations, especially as evolution selects for animals that are well adapted to specific environments. The speed of habituation of acoustic startle responses is in part determined by serotonergic neurons in the DRN [47] and dopaminergic neurons in the caudal hypothalamus [35]. These results were obtained by selectively breeding animals with extreme high or low habituation rates and comparing brain activity across generations, showing that evolution of behavior can act through changes in brain-wide neuromodulation. Such cross-generation studies may yield wider insights into how neuromodulatory systems collaborate to tune behavior within and across individuals. Zebrafish occupy an important position in such multi-regional physiology studies.

Non-neuronal and neuronal cell types for accumulation and experience-dependent state modulation

Behavioral state is also modulated due to accumulation of action outcomes in a behavior termed futility-induced passivity. When actions consistently fail to achieve their goals — for instance when the current is too strong to allow a fish to swim upstream — animals tend to switch their behavioral strategy or even become transiently passive. In zebrafish, this phenomenon has been studied in virtual arenas, where animals can be switched from a condition where swimming leads to simulated displacement to a condition where swimming is futile and no

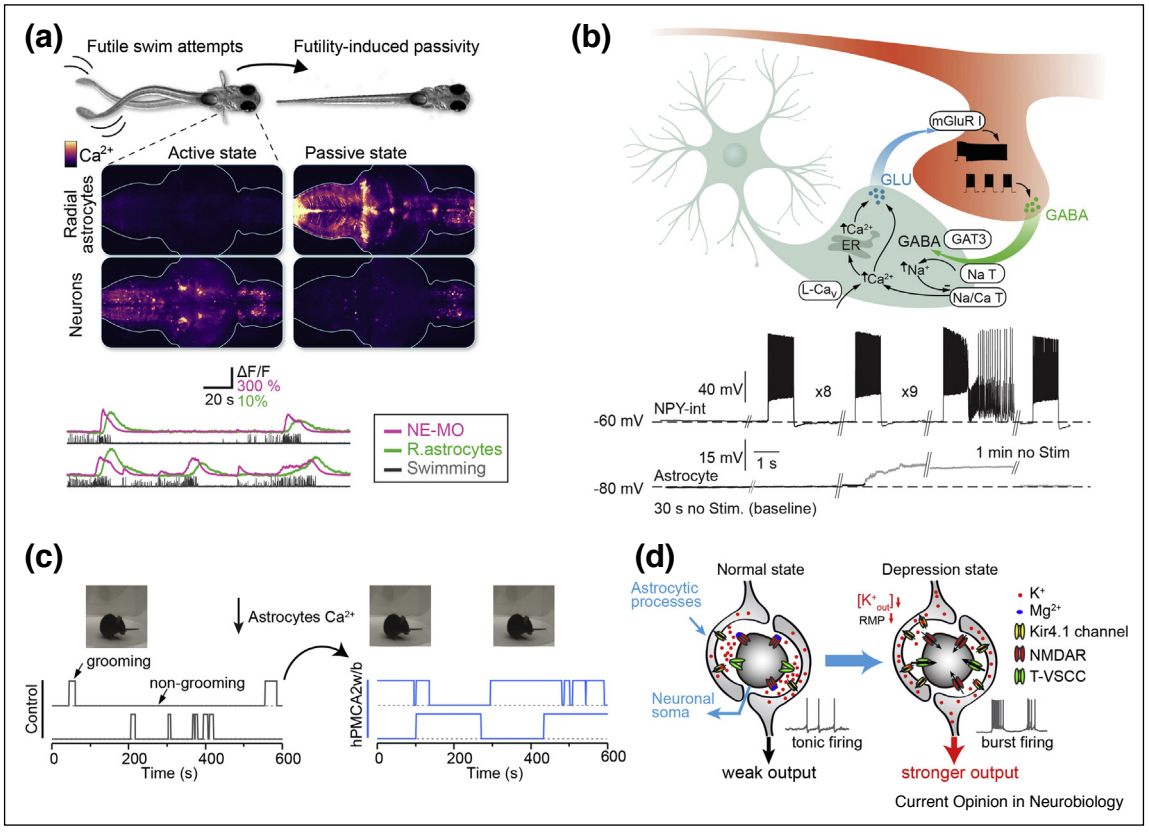
(d) DRN in zebrafish encodes an exploitation state and responds to successful hunts. From Ref. [40••].
(e) DRN stimulation in mice can prolong exploitation states. From Ref. [41].
(f,g) Sleep-promoting role of DRN in zebrafish and mice. Loss of function of DRN, achieved by preventing 5-HT synthesis in zebrafish and by genetic ablation in mice, reduced sleep in both species. From Ref. [44••].
(h,i) Functionally identified alertness-related cell types in zebrafish and mice. Alertness-related cells, whose activity correlates the reaction time from sensory cue to motor reaction, were identified by whole-brain, cellular-resolution registration of activity and molecular markers in zebrafish. Based on these results, the same cell types were imaged in mice and revealed consistent roles. From Ref. [46••].

longer affects the visual environment. Imaging from neurons and a glial cell type (radial astrocytes) showed that noradrenergic neurons detect swim bouts that do not lead to movement. Noradrenergic failure signals were integrated over many seconds by radial astrocytes (Figure 3a), followed by communication between these glia and nearby GABAergic neurons that triggered a passive behavioral state lasting many seconds. This work showed that astrocytes can play specific roles in neural computation and can cause shifts in behavioral state.

A similar mechanism may occur in mammalian circuits, where interplay between GABAergic cells and astrocytes has been observed (Figure 3b) [48••], potentially also representing a mechanism to switch networks to an inactive state. Responses to neuromodulators [49–52] and other interactions between astrocytes and neurons are seen across species, including in sleep [52,53], fear expression [50,51], obsessive-compulsive-like behavior (Figure 3c) [54], the baroreflex [55], depression (Figure 3d) [56], and seizures [57], demonstrating the potential of cross-species studies to reveal the role of glia in circuit modulation and behavior [58].

Understanding a related change in behavioral state, passive coping, has also benefited from the accessibility of the zebrafish brain. Passive coping is a strategy for animals to adaptively respond to consistent negative stimuli that are hard to overcome. In zebrafish, as in many mammals, a passive behavioral state can be evoked by exposing them to inescapable electric shocks [59,60]. Light-field and two-photon imaging revealed that this change in

Figure 3



Modulation of behavioral states via astrocytes.
(a) In zebrafish, radial astrocytes integrate norepinephrine released when motor action failed to cause sensory feedback. When such failure occurs repeatedly, calcium accumulates in radial astrocytes, which after about 20 seconds abruptly reduces swimming and triggers passive states. From Ref. [81••].
(b) Astrocytes integrate neuronal firing and drive long-lasting action potential trains (barrage firing) in GABAergic neurons. Left, repeated depolarization of a GABAergic neuron drives barrage firing, which correlates with depolarization of nearby astrocytes. Right, astrocyte activation is necessary (top) and sufficient (bottom) for causing barrage firing. From Ref. [48••].
(c) In mice, calcium decrease in striatal astrocytes increases self-grooming, a phenotype associated with obsessive-compulsive behavior. From Ref. [54].
(d) Astrocytes can modulate neuronal firing by changing the extracellular environment. In a depression model in rats, upregulating Kir4.1 in astrocytes hyperpolarizes habenula neurons, which then switch into bursting mode. From Ref. [56].

behavioral state was due to slowly increasing activity in the habenula, recruiting more neurons over time, with anatomical and functional connectivity to the dorsal raphe nucleus of the serotonergic system [59]. The acute antidepressant ketamine reduced both neuronal and behavioral phenotypes. In an analogous behavioral assay for rodents, activation of habenula neurons was causally linked to depressive states, and ketamine also reduced the activity of the habenula and the depression symptoms [56], suggesting evolutionary conservation of such pathways.

Thus far, we have discussed integrating environmental or experiential signals over prolonged periods in the context of behavioral states, but integrating purely sensory information over time is also ubiquitously important for animals. An example is making behavioral choices about which way to turn based on evidence of visual motion over multiple seconds, studied in fish using optomotor behavior [61,62] based on analogous sensory evidence accumulation studies in primates and rodents [63,64]. Fish watched randomly moving dots whose direction was biased to the left or the right, after which they made a behavioral choice to turn; turns were biased to the average direction of motion, but this choice was more delayed and more stochastic with higher uncertainty about visual motion direction. In both studies, whole-brain imaging showed accumulation of neural calcium signals in specific hindbrain nuclei reflecting the accumulation process. Although in mammals the entire distributed network for such tasks remains to be resolved, it seems likely that shared circuit mechanisms may operate across species.

Another circuit for integration of experiential signals over time was identified by light-field imaging [65•], using an operant conditioning task for head-embedded zebrafish as they learned to flick their tails left or right to switch off an aversive stimuli. This distributed network includes the cerebellum and an area called the ARTR [66] that together implemented the learned changes in tail movement. If we view cerebellar circuitry in different species as evolutionary instances of an ancient and evolving structure, this study is likely to inform broader theories of cerebellar function and its role in behavior.

Another set of connections between zebrafish and mammals that has fostered progress in systems neuroscience is in the neural basis of visual, chemical, and other types of threat avoidance. Neural circuits for visual and chemical avoidance behaviors include the superior colliculus in mammals [67,68] and the homologous region (optic tectum) in zebrafish [69–71], as well as the hypothalamus in both species [69,72–76]. The habenula, an area known to encode aversive experiences in mammals, has been shown to be required for updating learned place avoidance behavior in zebrafish [77].

Conclusions and perspectives

The studies described in this review illustrate recent and potential future contributions of zebrafish studies to systems neuroscience, including discovering the role of neuromodulation in changing behavioral states and the circuits and computations that lead to the recruitment of neuromodulatory centers. The examples also illustrate the feasibility and promise of multi-species comparison and understanding.

Neuroscientists are in search of a mechanistic and causal understanding of animal brains, which are supremely complex systems with connectivity across many spatial scales. Across species, progress is being made toward combining previously unattainable types of information, including millisecond spiking activity from hundreds of neurons across large brains [78,79], dense connectivity of small brains [26•,80], and whole-brain cell-identified activity in the transparent brains of larval fish [18•,81••,82–84]. Cross-species studies are important not only for taking into account that the species we observe are instances within the ongoing process of evolution, but also to allow neuroscientists to exploit the technological possibilities unique to each model organism, then relate the findings in that species to understanding derived from other species. Although our current understanding of neural circuit function is in many cases still approximate, through the combination of new data modalities, recording techniques, and cross-species comparisons, we expect to see ever more precise, quantitative, and predictive descriptions of the neural dynamics underlying behavior.

Conflict of interest statement

Nothing declared.

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- •• of outstanding interest

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