The highly pleiotropic nature of the flavonoid pathway suggests that even though white flowers are not producing anthocyanin pigments, many of the genes necessary to create color pigments are still deployed in other parts of the plant and to make other products. In this scenario, genes in the flavonoid pathway will not become nonfunctional due to relaxed purifying selection in white flowered plants. Perhaps the easiest path for evolution is to fix what is broken instead of further tinkering with a highly complex genetic pathway; this is exactly what seems to have occurred in *Petunia* (Figure 1). At some point in the past, selection favored white *Petunia* flowers and the broken *AN2* alleles spread in the ancestral lineage of the long-tube clade. After divergence between *P. exserta* and *P. secreta*, pollinator-mediated selection pressures changed and purple flowers were favored. The flavonoid pathway was ready to go — it just had to be turned on in the flowers and told to make color pigments. Although maybe improbable, a regain in function mutation in the broken *AN2* was actually a relatively easy fix to the problem. This newly functional allele, and a few expression changes in other key genes, allowed for the complete regain of floral pigment in the *Petunia* lineage. This story is a colorful reminder of how a seemingly implausible course for evolution may actually be a sensible path to adaptation.

REFERENCES


Balance Sense: Response Motifs that Pervade the Brain

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Measuring how the brain encodes and processes an animal’s own motion presents major technical challenges. New approaches demonstrate the viability and merit of measuring vestibular responses throughout the entire brain.

Vertebrates, from humans to fish, balance using the vestibular system. We move our bodies and limbs to stay upright, and our eyes and necks to steady vision. In doing so, we are making stabilizing movements based on information about posture — the orientation and conformation of our bodies. To detect instability, the inner ear — a.k.a. the vestibule — senses head movements [1]. The sensation of movement is then relayed to the hindbrain and, from there, throughout the nervous system (Figure 1A) [2,3]. A key goal in neuroscience is to define how the brain...
transforms sensed instability into corrective movements. To date, progress has been constrained by the twin challenges of stimulus presentation and data acquisition. Much of our current understanding of the vestibular system comes from a low-throughput technique, single-unit recordings, acquired during systematic movements of the animal [4]. Such investigations have informed nearly every aspect of systems-level neuroscience, for example multisensory integration [5] and motor learning [6]. But new techniques are needed to determine how populations of neurons collectively enable animals to balance. Two new studies reported in this issue of Current Biology by Favre-Bulle et al. [7] and Migault et al. [8] address this gap, demonstrating novel approaches for high-throughput mapping of vestibular responses throughout the brain.

Cutting-edge methods permit recording from every neuron in the brain using larval zebrafish. These fish afford a simple model of vestibular function as they use a single vestibular organ, the utricle, in each ear to enable both posture- and gaze-stabilizing reflexes [9,10]. Zebrafish can be genetically engineered to express a fluorescent reporter of intracellular calcium concentration throughout the brain. Changes in fluorescence that accompany neural activity can be measured with selective plane illumination microscopy (SPIM), using a thin sheet of light to illuminate the entire brain at a single depth (Figure 1B) [11]. By rapidly scanning the sheet, one can measure activity across the whole zebrafish brain multiple times each second. This approach has provided insights into how brains process sensory information in numerous modalities [12], but the challenges of head movement have precluded vestibular applications.

In this issue of Current Biology, two groups [7,8] report their use of novel SPIM apparatus to map vestibular responses in zebrafish. Migault et al. [8] miniaturized their microscope and mounted it on a motor, allowing them to rotate zebrafish during calcium imaging. Using this approach, they made whole-brain recordings while rotating the fish in the roll (barbecue) axis, thereby stimulating the utricles physiologically (Figure 1B, left). Favre-Bulle et al. [7] obviated the need to move the fish: instead, they used optical tweezers [13] to displace the utricle directly, as if it had been moved by a natural roll to the side (Figure 1B, right). They measured brain-wide responses to artificial stimulation of one or both utricles. Both groups found that their stimulation was sufficient to elicit balance reflexes that would stabilize the body and/or eyes. Together, these approaches provide means to understand how sensed imbalance from both ears is integrated and propagated throughout the brain.

To parse their data, both groups [7,8] devised analyses to categorize and map the types of neural responses to vestibular stimuli. The researchers segmented each SPIM imaging plane into putative neuron cell bodies and computed fluorescence time-series during stimulus presentation. Migault et al. [8] first addressed which regions responded as they rolled the fish sinusoidally, computing the amplitude and phase of responses throughout the brain. They also presented step stimuli, rapidly rolling fish to a tilted posture, and then performed regression to identify which areas were most strongly and linearly correlated with movement parameters — the posture and angular velocity of the fish. Favre-Bulle et al. [7] applied parametric stimulation of one utricle, and performed cluster analysis to define categories of excitatory and inhibitory responses. To understand how information from both utricles is combined, they stimulated each utricle separately, then mapped additive and subtractive responses to bilateral stimulation. In order to identify which
brain areas contained responsive neurons, both groups registered their data to the publicly available zBrain atlas [1]. Together, these recording and analysis techniques enabled the first, simultaneously acquired, brain-wide characterizations of vestibular responses at cellular resolution.

Migault et al. [8] found areas with excitatory responses to vestibular stimuli distributed throughout the brain, including in the telencephalon, habenula, thalamus, tectum, tegmentum, cerebellum, inferior olive, and throughout the medulla (Figure 2A,B) [8]. Intriguingly, they found that the majority of responsive neurons in most responsive brain areas were equally sensitive to steps towards both the left and right, with major exceptions found in the hindbrain (rhombomeres 6–7) and tegmentum, which favored contraversive roll, as well as the cerebellum, which favored ipsiversive roll (Figure 2A). Furthermore, Fourier analysis revealed that sinusoidal roll responses tiled stimulus phase. Neurons active in-phase with rightward or leftward angular velocity were most prominent in hindbrain vestibular nuclei (Figure 2B, ‘velocity’), while those in-phase with rightward or leftward posture were located nearer the midline throughout the midbrain and hindbrain (Figure 2B, ‘Posture’). Although these response maps do not exhaustively capture all neurons in the brain, and address only a small range of motion frequencies and magnitudes in a single axis, they reveal the striking prevalence and laterality of physiological vestibular responses.

By categorizing and mapping responses to non-physiological vestibular stimuli, Favre-Bulle et al. [7] discovered that different types of responses were segregated and widespread. From direct stimulation of a single utricle, the researchers described three categories of vestibular responses. The first category was the most common, comprising neurons relatively insensitive to weak stimuli; these neurons were found exclusively and bilaterally in the forebrain, including the telencephalon and habenula. The third response category, comprising neurons parametrically inhibited by utricle stimulation, occupied discrete patches in every region containing graded excitatory responses (Figure 2C). When both utricles were stimulated non-physiologically, simulating opposing rolls, some neurons exhibited additive responses — both excitatory and inhibitory — while others had activity suppressed. Interestingly, areas suppressed by conflicting stimuli (found in the midbrain, octavolateral hindbrain, and vestibulocerebellum) were observed by Migault et al. [8] to be direction selective for stepwise tilts [8], suggesting detection of rapid displacements may utilize information from both ears.

The contributions of these new studies [7,8] are unambiguous; it is now possible to measure brain responses to sensed instability rapidly and nearly comprehensively. These approaches in developing fish complement mammalian vestibular research, promising to reveal basic principles of vestibular function at the systems level. An important next step is therefore to relate utricle responses in zebrafish to those in mammals, including nonhuman primates [4]. Given the breadth of neurons with observed vestibular responses, a pressing question is how information propagates from vestibular nuclei in the brainstem. Measuring sequences of activation will require faster sampling than the 2.5–4 Hz employed here, which could be achieved by combining vestibular stimulation with new microscopy techniques, like SCAPE (swept, confocally-aligned planar excitation), that dramatically increase the spatiotemporal resolution of SPIM [15]. Relatedly, higher spatial resolution in depth will be required to achieve a complete account of every neuron in the brain.

These exciting new techniques can now be applied to long-standing questions about natural vestibular behavior. For example: how does the zebrafish brain use information about posture to control the timing and kinematics of stabilizing movements [9,16]? How are such balance commands conveyed to premotor areas and integrated with commands for voluntary locomotion [17]? How is direction tuning distributed across and segregated within brain areas, and are directional asymmetries in vestibular nuclei inherited by downstream areas [18]? In which areas are vestibular responses modulated by experience during early development [19]? How distributed is plasticity involved in vestibular reflex modulation [6,20]? The advances of Migault et al. [8] and...
Favre-Bulle et al. [7] will prove invaluable, as they open a new avenue towards discovering how stabilizing movements are controlled and are impaired in disorders of balance.

REFERENCES


Cell Cycle: Abrogating Interphase/M Phase Bistability

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A new study reports the ability to generate cells caught in a ‘no-man’s land’ between interphase and M phase by simultaneously disrupting feedback loops controlling the activities of the main mitotic driver Cdk1–cyclin B and its counteracting phosphatase PP2A-B55.

Microscopists in the 19th century were fascinated by cell division. Particularly striking was that relatively quiescent cells (i.e. those in a phase of the cell cycle that today we call interphase) began suddenly to exhibit new behaviors including shape changes, dissolution of the nuclear envelope, chromosome condensation, chromosome movements along the spindle apparatus, and (subsequently) cytokinesis. Equally remarkable, soon after the completion of these events (termed mitosis by Walther Flemming in 1882), the daughter cells reverted rapidly to apparent quiescence. A fascinating aspect of these early observations was the unidirectionality of these events. For example, once cells started to exhibit mitotic properties, they did not again become quiescent until cell division was complete. In a new study reported in this issue of Current Biology, Rata et al. [1] explain the molecular basis of this unidirectionality and use this information to establish conditions under which cells can go backwards to their previous state without finishing mitosis. We now know that the transitions between interphase and mitosis...