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Figure 1. The multifaceted role of plant vacuoles.
Plant vacuoles perform an incredibly diverse array of cellular functions. Concept and realization in collaboration with diogoguerra.com.

by sequestering them into the vacuole. This way, toxic ions are kept out of the cytoplasm where they could inhibit enzyme function or scavenge important nutrients. Detoxification mechanisms of toxins and xenobiotics are very similar in animals and plants, but while animals can excrete, plants use an ‘internal excretion’ and store the modified toxins in the vacuole.

But it’s not just their soil and water that plants cannot escape — bacteria, fungi, viruses and herbivores may constantly attack it. Vacuoles store a great number of secondary metabolites to repel and deter such attackers. Many of the natural defence compounds that plants store in vacuoles for their own good are useful for pharmaceutical purposes. Two examples are the alkaloids vinblastine and morphine. Vinblastine is used commercially as a mitosis-inhibitor during chemotherapy of cancer patients. Together with its related alkaloid vincristine, also used in cancer therapy, it occurs in large quantities in vacuoles of special cells (idioblasts) of the madagascan plant rosy periwinkle (*Catharanthus roseus*). Morphine is well known for its pain-relieving function. Its synthesis occurs in different cell types and is finally stored in small, special vacuoles present in the laticifers of opium poppy.

Why are plants able to grow so large upright against gravity? In part, because of their vacuole. In adult cells of leaves, roots and stems the plant vacuole occupies 80–90% of the cell volume. Vacuoles accumulate osmotically active solutes causing water influx and cell swelling. The resultant hydrostatic pressure against of the rigid cell wall constitutes turgor pressure, which plants need to grow against the gravitational force to reach a large size and optimize photosynthesis. To save energy, the osmotically active solutes stored in the vacuole are only rarely organic solutes and mostly inorganic anions such as sodium, potassium or chloride. If plants suffer from a lack of water in the soil, cell turgor decreases and plants start wilting.

Where can I find out more?

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Primer

Whisking

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Eyes may be ‘the window to the soul’ in humans, but whiskers provide a better path to the inner lives of rodents. The brain has remarkable abilities to focus its limited resources on information that matters, while ignoring a cacophony of distractions. While inspecting a visual scene, primates foveate to multiple salient locations, for example mouths and eyes in images of people, and ignore the rest. Similar processes have now been observed and studied in rodents in the context of whisker-based tactile sensation. Rodents use their mechanosensitive whiskers for a diverse range of tactile behaviors such as navigation, object recognition and social interactions. These animals move their whiskers in a purposive manner to locations of interest. The shapes of whiskers, as well as their movements, are exquisitely adapted for tactile exploration in the dark tight burrows where many rodents live. By studying whisker movements during tactile behaviors, we can learn about the tactile information available to rodents through their whiskers and how rodents direct their attention. In this primer, we focus on how the whisker movements of rats and mice are providing clues about the logic of active sensation and the underlying neural mechanisms.

Whiskers

Most land and aquatic mammals use mechanosensitive whiskers to touch and explore objects of interest. Whiskers are specialized hairs emanating from follicles that are densely packed with nerve endings. Mechanical interactions between a whisker and an object are converted by the whisker into stresses in the follicle. Mechanoreceptors in sensory neurons transduce these stresses into neural signals, which are interpreted by the central nervous system to learn about the structure of the world.

The whisker shape plays a critical role in translating mechanical stimuli to neural excitation. Across species, whiskers have diverse shapes, which reflect the behaviors and ecological niches of their owners. For example, the

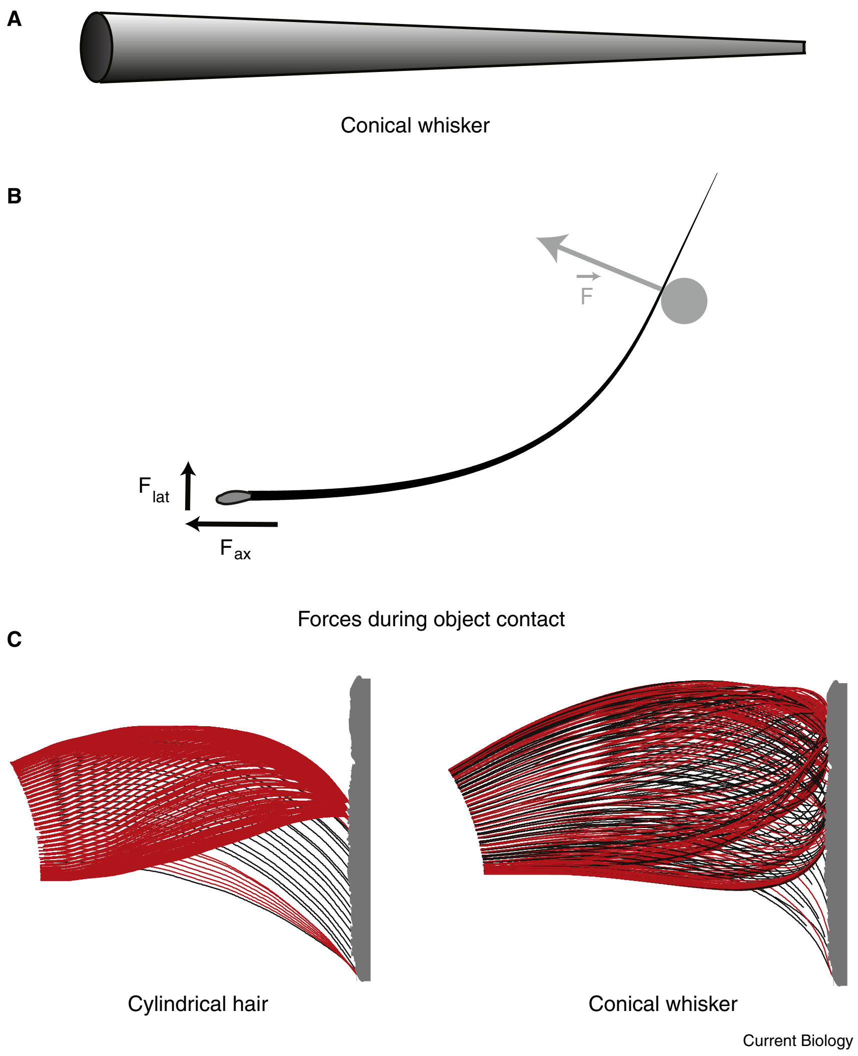

Figure 1. Rodent whiskers are conical.
(A) Schematic of a rodent whisker. Whiskers are almost perfect cones, with a diameter of 100 micrometers at the base and 3 micrometers at the tip (reproduced from Hires *et al*. 2013). (B) Whisker interacting with an object (grey circle). The object exerts a force F on the whisker, normal to the whisker. Depending on the location where the object is applied to the whisker the forces will be decomposed into different axial (F_{ax}) and lateral (F_{lat}) components at the follicle (based on Hires *et al*. 2013). (C) Time-lapse sequence showing a tracked human hair (left) or a mouse whisker (right) interacting with a rough surface (grey). Red traces correspond to time points when the whisker tip is trapped, whereas black traces correspond to moments when the whisker is sliding along the surface. Conical whiskers sweep along a rough texture with characteristic stick-slip micromotions (right). In contrast, a cylindrical hair gets stuck (left) (reproduced from Hires *et al*. 2013).

whiskers of harbor seals have elliptical cross sections with an undulating diameter. This structure enhances the seal's ability to analyze fine-scale water currents associated with prey movements.

The main facial whiskers of rodents, also called macrovibrissae, have almost perfect conical shapes (Figure 1A). A typical rodent whisker has a diameter of 100 micrometers at its base near the follicle. The whisker narrows gradually to only a few micrometers at the tip, about twenty millimeters from the base. This conical shape allows rodents to measure object distance using a single whisker and prevents the whiskers from getting caught in tight spaces.

A conical whisker can serve as a kind of mechanical ruler for object distance. The bending stiffness of a whisker, defined as the bending moment required to produce a given change in whisker curvature, is proportional to the fourth power of the whisker diameter. The bending stiffness therefore decreases by five orders of magnitude from the base to the tip, reflecting the decreasing diameter of the conical whisker. This means that a force applied to the whisker by touch causes dramatically more bending when applied at the tip compared to the whisker base. The bending provides salient cues about the location of the touched object along the whisker (Figure 1B). For example, the larger bending at the tip causes a larger ratio of the axial component to the lateral component of the force at the follicle. Behavioral experiments show that rodents can do vector math with their face: they interpret the relative amplitudes of axial and lateral force vectors to extract the location of touch along the whisker.

The conical whisker shape is critical for sweeping whiskers along rough surfaces, with implications for the exploration of textures and the management of whiskers in tight spaces. Hypothetical cylindrical whiskers (or human hair) would get stuck in small surface imperfections (Figure 1C). In contrast, the tips of conical whiskers easily slide over surfaces because the relatively stiff whisker base can pull the wispy tips out of traps in rough textures.

Rodents have thirty-five such whiskers (Figure 2A), which are arranged in a grid on the face and given names similar to the squares on a chessboard. Individual whiskers have specific lengths and thicknesses, with longer whiskers more caudal and shorter whiskers more rostral. When a whisker is shed, as they are prone to do every couple of months, a new whisker regrows in its place with essentially identical dimensions, highlighting the importance of whisker shape.

The whisker grid represents the front end of labeled lines, one line per whisker, where tactile information from each whisker is mapped to topographically arranged groups of neurons throughout the brainstem, thalamus, and somatosensory cortex. A large area of the rodent neocortex, called the barrel cortex because of its distinctive cytoarchitecture, is devoted to processing tactile information from the whiskers, which highlights the importance of whisker-based touch as a sensory modality for these animals. In the barrel cortex, columns of 10,000 neurons process information primarily from individual whiskers. The columns are arranged in a map reflecting the arrangement of whiskers on the face.

Whisker movements

Larger animals, such as cats and seals, move their whiskers via head movements and also with extrinsic muscles that move the entire whisker pad forward and backward with respect to the head. Many rodents in addition have a specialized system of intrinsic muscles, one per whisker, for fine-scale control of whisker movements. Both extrinsic and intrinsic muscles cause the whiskers to pivot forward and backward around the follicles. Therefore, whiskers move primarily in an arc in the rostral-caudal direction. This complex musculature allows fine-scale positioning of the entire group of whiskers and individual whiskers during tactile exploration, similar to digits on the human hand. Also similar to human digits, the motor neurons driving whisker movements can be directly accessed by the motor cortex for high-level control of whisker position.

Rats and mice display a diverse repertoire of innate and learned whisker movements that allow them to gather specific types of information. Insights about whisking strategies are based on time-lapse images of whiskers during behavior. Because whiskers move at considerable speed (up to 10,000 degrees per second) they must be imaged using high-speed (1,000 frames per second) videography. The vast number of images collected in these measurements (millions per hour) places severe demands even on advanced computational infrastructures and requires fully automated image analysis using efficient algorithms. There are now several powerful software packages that reliably track a subset of whiskers over time and during interactions with objects. These algorithms output the whisker position and shape at each time-point.

This hard-won information is extremely valuable. The position of the whisker base with respect to the head reflects the activation of motor neurons, and hence the motor programs that underlie active sensation. The deformation of whisker shape by touch (Figure 1B) provides almost complete information about the forces in the sensory follicles.

When exploring novel environments, rodents move their whiskers forward (protraction), and rhythmically modulate whisker position about this protracted position at a well-defined frequency (15–20 Hz mice, 5–10 Hz rats) continuously over dozens of cycles (Figure 2B). Rhythmic whisking is coupled to running: the faster rodents run, the faster they whisk and the further they protract their whiskers (Figure 2B,C). When turning, rodents whisk in a bilaterally asymmetric fashion, so that they explore the space they are about to enter in an anticipatory manner (Figure 2D). The whisker positioning and movement thus reflect a strategy to increase the time available for moving rodents to detect, analyse and navigate obstacles.

As rodents become familiar with an environment, the amplitudes of whisker movements during running decrease, possibly indicating confidence that they will not encounter unexpected objects. Conversely, in environments containing frequent and unpredictable obstacles, whiskers protract even further. These observations show whisker movements are under high-level neural control. Conversely, measurements of whisking can be used to gauge the animals' expectations about the environment.

Head-fixed experiments have become the 'gold standard' in the exploration of neural mechanisms underlying behavior. Head-fixation provides a high degree of stimulus control (for example, the tactile stimuli within reach of the whiskers) and behavioral read-out (for example, whisker position). Head-fixation is also useful in the analysis of tactile behaviors, because whiskers and their interactions with objects can be tracked continuously over long behavioral epochs. In contrast, in freely moving rodents whisker video is limited to an occasional few seconds at a time.

Head-fixed mice whisk naturally when running on a spherical treadmill. Measurements of whisking in head-fixed rodents have been combined with fine-scale measurements of locomotion and sniffing, and with recordings and manipulations of neural activity. These have revealed that rhythmic whisking is phase-locked to stepping, sniffing, and even to ultrasonic vocalizations, likely through neural circuits in the brainstem; such synchronization might implement a common time base for neural computations.

Interactions with objects

During exploration and tactile discrimination, rodents palpate objects with their whiskers (Figure 3A,B). Touch modulates whisking over multiple time scales. Even during the first whisking cycle containing touch (<100 ms), rats adjust their whisker trajectory to prolong the protraction and press their whiskers further into an object. On subsequent cycles, the whisking amplitude on the side of the object is reduced, whereas the whisking amplitude on the opposite side is increased (Figure 3C). This orienting towards objects allows rats to bring a larger number of whiskers into light contact with the object. In general, feedback between whisker movement and touch allows for integration of

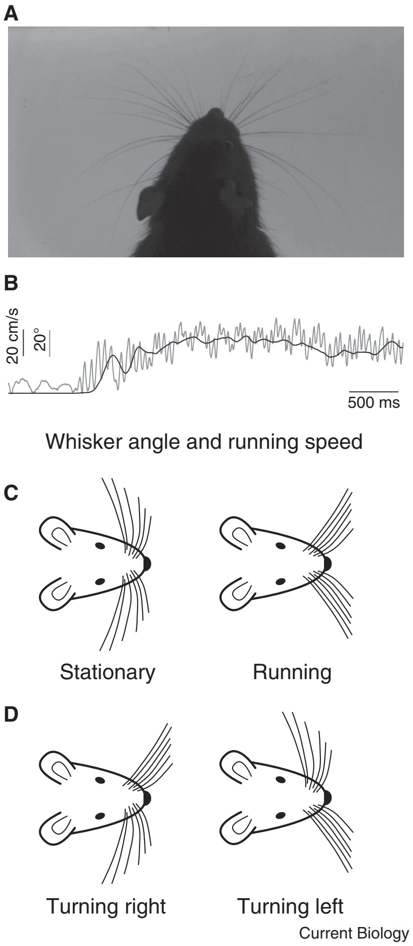

Figure 2. Whisker movements during exploration.

(A) One frame in a high-speed video showing a rat head and its whiskers (reproduced from Grant *et al*. 2008). (B) Running speed (black) and whisker angle (grey). Faster running corresponds to more protracted whisker positions. The slow (4 Hz) oscillation in running speed corresponds to strides and is locked one-to-one to a slow oscillation in the whisker angle (reproduced from Sofroniew *et al.* 2014). (C) When running, rodents protract their whiskers (see also B). (D) When turning, rodents position their whiskers in a bilaterally asymmetric fashion. The space that the animals are about to enter is explored in an anticipatory manner.

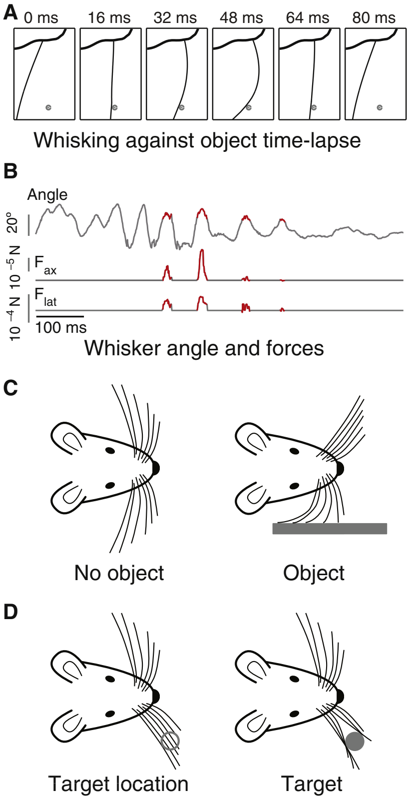

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Figure 3. Interactions of whiskers with objects.
(A) Time-lapse sequence showing one whisker touching a pole. After touch, the whisker is bent by forces exerted by the object on the whisker (see Figure 1B; reproduced from Pammer *et al.* 2013.) (B) The whisker angle (top) and forces applied to the whisker follicle by object contact (bottom). Periods of touch are colored in red (reproduced from Pammer *et al*. 2013). (C) Rodents use their whiskers to track walls, and can adjust their whisking amplitude in a bilaterally asymmetric fashion following object contact. (D) Rodents direct their whiskers to locations where they expect an object of interest.

information across multiple touches, which is critical for exploration of object shape and improving perceptual accuracy during decision-making.

Interactions between whiskers and walls have been studied during navigation in a whisker-based, tactile virtual reality system, where head-fixed mice run on a spherical treadmill and interact with walls that move in closed-loop with the movements of the treadmill. This allows the simulation of a tactile corridor. Immediately after encountering this virtual corridor, mice track the walls without training. The forces exerted by the walls on the whiskers predict movement with respect to the walls, suggesting that mice monitor these forces during navigation in dark corridors.

Beyond natural behaviors, mice have also been trained on tactile tasks designed to isolate specific sensory features. In active sensation, animals are free to move their sensors to optimize sensory information. Mice adopt distinct purposive whisking strategies to solve specific tasks. For example, in an object location discrimination task, a pole transiently comes within reach in one of two locations, predicting reward in one of two reward ports. To solve this task, mice learn to move their whiskers in short bouts when the object is present (Figure 3A,B); they preferentially engage one of the object locations in an apparent attempt to maximize whisker touch with one of the two pole locations (Figure 3D). At the neural level, this strategy maximizes the spike count difference in barrel cortex neurons across the pole locations. The spike count underlies perception of object location. Using this learned whisking strategy, mice can discriminate object locations separated by only one degree of whisker arc.

Rodents can discriminate texture roughness with their whiskers, with sensitivities that rival the abilities of human digits. To explore surface textures, rodents sweep their whiskers over surfaces. The whisker tips transiently stick to surface traps, followed by high-velocity slips. The conical whisker shape is critical for rapid stick-slip sequences. The slips are encoded in the barrel cortex by sharp spike volleys. The pattern of these irregular stick-slip patterns, and the resulting spike trains, provide rich information about the surface structure.

Conclusions

Sensation is an active process: in vision, saccades direct gaze to regions of interest; in olfaction, sniffs bring air into the nasal cavity; in somatosensation, whiskers or digits are moved over objects. The whisking strategies of rodents have now been deciphered in innate and learnt behaviors. Whisker movements reflect the animal's expectations and provide clues about the salient tactile features. A comprehensive understanding of how tactile perception arises at the level of individual whiskers within brief epochs of time is coming into view.

However, a Gestalt of the tactile world is ultimately based on information integrated across multiple whiskers in space and over multiple touches in time. Rodents must decode this information, including whisker position and forces in the whisker follicle, to simultaneously determine multiple object features, such as object shape and texture. We still know little about how such complex touch-based perception is achieved. A careful analysis of whisker movements during complex tactile behaviors will provide an important entry point to deciphering these cognitive processes.

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