

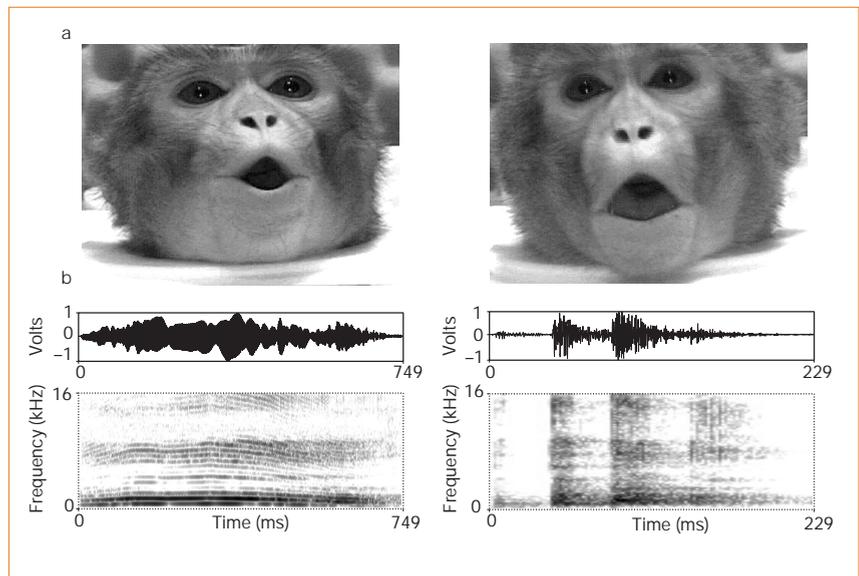
# Facial expressions linked to monkey calls

Pulling a face to emphasize a spoken point is not seen as just a human prerogative.

The perception of human speech can be enhanced by a combination of auditory and visual signals<sup>1,2</sup>. Animals sometimes accompany their vocalizations with distinctive body postures and facial expressions<sup>3</sup>, although it is not known whether their interpretation of these signals is unified. Here we use a paradigm in which 'preferential looking' is monitored to show that rhesus monkeys (*Macaca mulatta*), a species that communicates by means of elaborate facial and vocal expression<sup>4-7</sup>, are able to recognize the correspondence between the auditory and visual components of their calls. This crossmodal identification of vocal signals by a primate might represent an evolutionary precursor to humans' ability to match spoken words with facial articulation.

We tested whether rhesus monkeys could recognize auditory-visual correspondence between their 'coo' and 'threat' calls (Fig. 1a). These are the most frequently produced calls in the rhesus monkey repertoire, both in the wild and in captivity. Coo calls are long, tonal signals (Fig. 1b) and are produced in an affiliative context<sup>5</sup>; threat calls, by contrast, are noisy, pulsatile calls of short duration (Fig. 1b) and are produced during agonistic encounters<sup>5</sup>. Each of these calls is accompanied by a unique facial gesture: coo calls are associated with a small mouth opening and protruding lips, whereas threat calls are conveyed with a larger mouth opening and no lip protrusion<sup>6,7</sup> (Fig. 1a).

To test the monkeys, we adopted a preferential-looking technique that has been used to test crossmodal speech perception in prelinguistic human infants<sup>8,9</sup>. Subjects were seated in front of two liquid-crystal-display monitors and shown two side-by-side digital 2-s videos (synchronized to the onset of the audio track), played in a continuous loop for 1 min, of the same conspecific individual ('stimulus animal') articulating the two different calls. A sound track that corresponded to one of the two facial postures was played through a speaker located behind and between the two monitors; a curtain concealed all equipment apart from the screens and the camera lens. Each trial began when the subject fixated his gaze towards the camera, and was scored by two observers who were blind to the conditions and who judged, frame by frame, whether the subject was looking towards the right screen, the left screen or away from both (inter-observer agreement was 0.98, as measured by Pearson's correlation).



**Figure 1** Multisensory integration by rhesus monkeys of their 'coo' and 'threat' calls. **a**, Single video frames of facial gestures made during coo (left) and threat (right) calls, shown at the point of maximal mouth opening. **b**, Oscillograms (top) and spectrograms (bottom) of the coo and threat vocalizations: coo calls are long and tonal; threat calls are short, pulsatile and noisy.

We used two separate stimulus sets with two different stimulus animals to ensure that recognition was not based on an idiosyncratic property of the vocalizer. The call played through the speaker, the left-right position of the two facial gestures, and the stimulus sets were counterbalanced. The dependent measures used were the percentage of total looking time to the match video, and the duration of the longest single look at each of the two screens.

We predicted that, if rhesus monkeys recognized the correspondence between the heard sound and the appropriate facial posture, then they would spend more time overall looking at the match video. We found that the mean percentage of looking time devoted to the match was 67.3%, which was significantly greater than the 50% chance value ( $t = 4.19$ ,  $d.f. = 10$ ,  $P = 0.002$ ). Furthermore, all 11 subjects looked for longer at the match face (binomial test,  $P < 0.001$ ). There were no significant interactions between the time spent looking at the match face and the stimulus set or the position (left versus right) of the monitor. Subjects did, however, look for longer when the match face was articulating the coo call than when the match face was making the threat call ( $F = 18.75$ ,  $d.f. = 10$ ,  $P = 0.002$ ). A separate analysis revealed that preferences for looking to the match screen were still significant for coo calls alone ( $t = 6.20$ ,  $d.f. = 4$ ,  $P = 0.003$ ) and threat calls alone ( $t = 2.98$ ,  $d.f. = 5$ ,  $P = 0.03$ ). Rhesus monkeys' longest

single fixations were, on average, longer for the match (mean  $\pm$  s.e.m.,  $7.97 \pm 1.16$  s) than for the non-match face ( $4.26 \pm 0.98$  s). This difference was significant ( $t = 3.94$ ,  $d.f. = 10$ ,  $P = 0.003$ ). Furthermore, 10 of the 11 subjects had longer single fixations to the match face (binomial test,  $P = 0.006$ ).

Our results indicate that rhesus monkeys have an inherent ability to match acoustically presented conspecific vocalizations with the appropriate facial posture. The pattern of our results closely follows those for crossmodal speech recognition by prelinguistic human infants using the same preferential-looking paradigm<sup>8,9</sup>. However, to our knowledge, this is the first demonstration of auditory-visual integration in an animal vocal communication system. We interpret the preferential fixations on the sound-specified facial expression as evidence that rhesus monkeys detect information that is invariant across visual and acoustic displays of vocal expressions. The presence of multimodal perception in an animal's communication signals may represent an evolutionary precursor of humans' ability to make the multimodal associations necessary for speech perception<sup>10,11</sup>.

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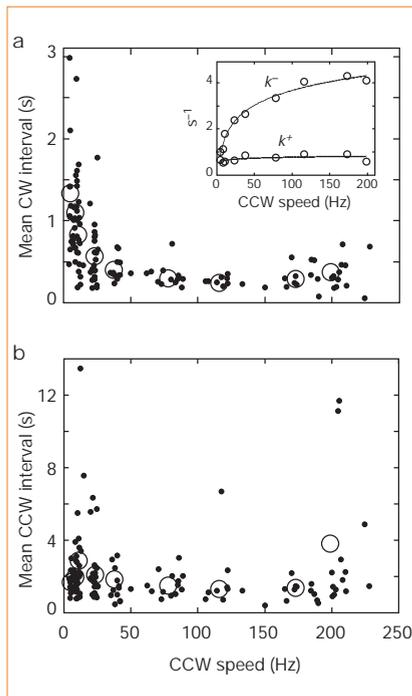
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Biomechanics

## Bacterial flagellar switching under load

Flagellated bacteria swim up gradients of chemical attractants by modulating the direction of rotation of their flagellar motors, but how this switching mechanism works is not understood. Here we show that the probability of the motor rotating in the clockwise direction increases under high load, when the motor spins slowly (at less than 50 Hz). We suggest that either the switch is responding to small changes in torque — the torque increases only fractionally between 50 Hz and stall — or it senses motor speed, perhaps by means of proton flux.



**Figure 1** Behaviour of the flagellar switch of *Escherichia coli* under different loadings. **a**, **b**, Mean intervals for clockwise (CW; **a**) and counterclockwise (CCW; **b**) rotation of the flagellar motor, plotted as a function of mean CCW speed. Filled symbols, values measured for each cell; hollow symbols, averages over the ensemble of cells for beads of a given size, with the values for each cell being weighted equally. Bead diameters were 0.36, 0.54, 0.74, 1.03, 1.20, 1.44, 1.79, 2.13 and 2.60  $\mu\text{m}$  (from right to left); the number of cells studied for each of these bead sizes was 12, 12, 7, 12, 18, 18, 16 and 10, respectively. Each bead was monitored for 5 min at 20 °C. Inset, rate constants  $k^- = 1/(\text{mean CW interval})$  and  $k^+ = 1/(\text{mean CCW interval})$  were computed for each cell and then averaged over the ensemble of cells for beads of a given size, with the values for each cell being weighted equally.

Flagellar filaments of an *Escherichia coli* sticky-filament mutant<sup>1</sup>, which is otherwise wild-type for chemotaxis, were shortened by viscous shear, and cell bodies were fixed to a polylysine-coated glass coverslip. Latex beads of various sizes were adsorbed to filament stubs and their rotation was followed in a weak optical trap with a quadrant detector<sup>2</sup>.

Figure 1 shows clockwise and counterclockwise intervals for rotation plotted as a function of counterclockwise speed, together with rate constants for transitions between the clockwise and counterclockwise states. Clockwise intervals lengthened appreciably at high loading ( $k^-$  decreased), whereas counterclockwise intervals remained about the same for all loads ( $k^+$  remained roughly constant).

A similar asymmetrical performance is seen under the action of fumarate<sup>3</sup>. However, when an attractant is added, the cells respond by shortening their clockwise intervals and lengthening their counterclockwise intervals<sup>4</sup>. From the results shown in Fig. 1, it follows that a motor that is spinning rapidly, as motors do in swimming cells, will spend a larger fraction of its time turning counterclockwise and will change direction more frequently than a motor that is spinning slowly, as motors do in tethered cells.

In a wild-type cell, the direction in which the motor spins depends on the degree of phosphorylation of a small signalling protein, CheY, which is activated by a kinase that is coupled to the chemoreceptors<sup>5</sup>. To determine whether this signalling pathway is involved in the load response, we repeated a number of measurements using cells in which this pathway had been disrupted. We used a strain in which *cheY*, *cheA* (which encodes the kinase) and *cheZ* (which encodes a phosphatase) were all deleted and which expressed CheY<sup>13DK106YW</sup> (CheY\*\*), a variant with fixed activity<sup>1</sup>. The response to different loadings was the same as that of wild-type cells, indicating that the motor's sensitivity to load is probably not due to feedback from the chemotaxis signalling network, but is instead an inherent property of the motor.

The bacterial flagellar motor is powered by protons moving down an electrochemical gradient and has a distinctive torque–speed relationship. At 20 °C, the relative torque falls gradually from unity at stall to about 0.9 at 120 Hz, and drops rapidly to zero at about 260 Hz (ref. 6); in the latter regime, the torque decreases

markedly at lower temperatures or when H<sub>2</sub>O is replaced by D<sub>2</sub>O (ref. 7), indicating that the movement of mechanical parts and/or of protons becomes rate limiting.

We found switching to be particularly sensitive to load only during high-torque, low-speed operation, and mainly at speeds of less than about 50 Hz (Fig. 1). Over this speed range, the torque changes by about 5%. There is evidence that each revolution of the motor requires the passage of a fixed number of protons<sup>8</sup>. So, rather than sensing changes in torque, might the switch be monitoring proton flux?

Flagellated bacteria are sensitive to a variety of environmental factors, including mechanical stimuli. Swimming in viscous solutions or near surfaces can trigger developmental changes — for example, swarmer-cell differentiation in *E. coli*<sup>9</sup> or lateral flagellar synthesis in the marine organism *Vibrio*<sup>10</sup>. In the latter case, changes in gene expression occur in response to exposure to sodium-channel blockers, indicating that they might be caused by a reduction in motor ion flux<sup>11</sup>. It is possible that these different responses rely on a common mechanosensory mechanism.

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erratum

Rapid change in mouse mitochondrial DNA

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There is an error in the Volo Bog and Glenview components of Fig. 1 of this communication: two *Mw* haplotype mice were shown in the 1950–99 column of the former that should have been in the 1950–99 column of the latter. This mistake does not affect the conclusions, as the analysis was done with mice from the correct category. Sample sizes (left and right columns, respectively) for the different locations were: Volo Bog, 19 and 41; Glenview, 5 and 6; Illinois Beach, 8 and 5; Highland Park, 2 and 5; Palos, 1 and 8 (note that sample numbers were inevitably low for museum specimens).