Jays that had previously interacted with one of the birds they observed drew inferences about their rank relative to the demonstrator, and showed a graded, quantitative response based on their observations. Jays that observed very similar interactions, but had never interacted directly with any of the birds they observed, failed to show either effect. This pattern rules out alternative general explanations, such as badges of status[19] or dispositional responses to seeing another bird win or lose[15–17]. This work constitutes a direct demonstration of transitive inference in social settings, and supports the hypothesis that social complexity provided a crucial context for the evolution of cognitive abilities. □

Methods

Test procedures

To familiarize the birds with the apparatus, each jay was placed alone in one of the end compartments of the encounter chamber. After 30 s the dividers were raised and the bird was allowed to explore the apparatus until it discovered and consumed a peanut. Each bird received six such familiarization trials before beginning staged encounters. During staged encounters (Fig. 1a), each member of a dyad was initially placed in one of the end chambers (randomly selected). After 10 s the opaque divider was lifted, providing visual contact between dyad members through the second, transparent divider. After an additional 10 s the transparent divider was lifted, giving the birds simultaneous access to the central contest area. To facilitate recognition of individuals for video scoring, one of the dyad members in each encounter was marked on the wing primaries with water-soluble white paint. After the encounter the paint was removed.

Group formation and selection of pairs for testing

Our experimental design required sets of birds of relatively similar rank who were unknown to each other, but whose relative dominance could be predicted accurately. We first divided the birds into three groups; two groups of six birds and one group of four. The small size of the groups minimized the possibility of nonlinear relationships. Once the within-group hierarchies were established, we then determined eight of the 32 possible cross-group dominance relationships by pairing the second-, third- and fourth-ranked birds in group 1 with those of the same rank in group 2, and the second- and third-ranked birds in group 3 with the second- and third-ranked birds in both groups 1 and 2. The outcomes of these within- and cross-groups dominance encounters were subsequently used to select sets of observers and demonstrators.

During exhibition sessions the demonstrator was paired with two other birds, one dominant and one subordinate to the demonstrator. In experimental conditions one of these other birds had to be a stranger to the observer and the other a known dominant. In control conditions both birds had to be strangers to the observer. In addition, because the experimental design required birds that could both win and lose encounters with members of their group, birds at the top or bottom of their group hierarchies were not used as observers. These constraints limited the number of possible pairings that could be generated, with the result that some individuals were used in more than one trial. Nine observers and six demonstrators participated in the six experimental and six control pairings.

To control for prior experience in winning and losing, we arranged daily maintenance encounters between each of the demonstrators and observers and members of their own groups. During the three weeks before testing, each observer had an average of 15 encounters with five other birds, of which he won 47%; for the four observers that were tested more than once, at least two months passed between successive trials. In the same time period, each demonstrator had an average of 16 prior encounters with four other birds, of which he won 56%; for the three demonstrators tested more than once, at least 10 days passed between successive trials.

Behavioural indices

Because display behaviour is often a more reliable indicator of dominance than gaining access to food[20], we used relative frequencies of behavioural acts to assess dominance. To obtain an empirically valid index of relative dominance in which the contributions of the different behavioural events were appropriately weighted, we first calculated (for each individual in each encounter) the difference between the raw counts of dominant and subordinate actions divided by their sum. Differences between dyad members in the value of this ratio, which weighted all action patterns equally, provided an initial approximate measure of relative dominance. From the 36 within-group dyads in the study, we extracted a set of 15 exemplars, dyads in which the mean of this ratio (averaged over all six encounters) was larger than 0.5 and in which one of the dyad members consistently dominated in all six encounters. Because some behaviours are better indicators of social status than others, however, a simple sum of event frequencies is often misleading as an indicator of relative dominance. To obtain a more sensitive measure, we subjected the raw counts from the last three encounters from each exemplar to canonical discriminant analysis[18], which produces the weighted linear combination of standardized variables that best distinguishes between data classes. In the final configuration, three variables—the frequencies of stare at and look away, and the sum of the frequencies of the three other submissive displays—were log-transformed, standardized and combined into two weighted discriminant functions that constituted dominance and subordinance indices. The difference between dominance and subordinance provided a direct measure of each individual's relative social status (relative social status = dominance − subordinance), and in this combination the discriminant functions correctly categorized 93% of the encounters in the exemplar data set.

Received 7 April; accepted 3 June 2004; doi:10.1038/nature02723.

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Acknowledgements We thank N. Howe and E. A. Simpson for assistance in data collection and B. Luke Stafford for help in designing Fig. 1. Supported by University of Nebraska Research Enhancement Funds and an NSF grant to Northern Arizona University.

Competing interests statement The authors declare that they have no competing financial interests.

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Complex auditory behaviour emerges from simple reactive steering

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The recognition and localization of sound signals is fundamental to acoustic communication[1,2]. Complex neural mechanisms are thought to underlie the processing of species-specific sound patterns even in animals with simple auditory pathways[3,4]. In female crickets, which orient towards the male's calling song, current models propose pattern recognition mechanisms based on the temporal structure of the song[5–7]. Furthermore, it is thought that localization is achieved by comparing the output of the left and right recognition networks, which then directs the female to the pattern that most closely resembles the species-specific song[8–10]. Here we show, using a highly sensitive method for measuring the movements of female crickets, that when walking and flying each sound pulse of the communication signal releases a rapid steering response. Thus auditory orientation emerges from reactive motor responses to individual sound

pulses. Although the reactive motor responses are not based on the song structure, a pattern recognition process may modulate the gain of the responses on a longer timescale. These findings are relevant to concepts of insect auditory behaviour and to the development of biologically inspired robots performing cricket-like auditory orientation[11–13].

Many animals, including frogs and insects, communicate with stereotyped patterns of constant frequency sound pulses[14], making them suitable models to investigate the neuronal mechanisms underlying acoustic pattern recognition and localization[15,16]. Male crickets (*Gryllus bimaculatus*) produce long-lasting calling songs, made up of chirps of four to five sound pulses[17,18] that are repeated at 2–3 Hz. Female crickets are attracted by this calling song and walk[4,19] or fly[20,21] towards singing males. Auditory processing takes place in a simple pathway[15,22]. When exposed to artificial songs, females perform phonotactic walking on trackballs, which allows quantitative analysis of their behaviour[23–25]. With such experiments, pattern recognition is inferred from the animal's auditory orientation[4]. Previous investigators relied on high inertia trackballs and monitored the animal's walking speed averaged over 500–1,000 ms. Here we used a new, highly sensitive trackball (Fig. 1a), which measured the path of tethered stationary walking crickets with a temporal resolution of 0.3 ms (see Methods). Modulations of walking speed by individual steps could be recorded (Fig. 1b) and it revealed so far undetected rapid steering movements.

Auditory pattern recognition in female crickets is tuned to the species-specific song structure and may be achieved by template matching[5], temporal band-pass filtering[6] or cross-correlation analysis[7]. To gain insight into auditory orientation, we analysed the steering behaviour to split-song patterns[26]. A chirp with six sound pulses (duration 21 ms, interval 21 ms) was split so that every two consecutive pulses were presented from opposite sides (Fig. 2a, b; top) at $\pm 45°$ from the animal's longitudinal axis. At the onset of sound, the animals started oriented walking with a speed of 5–7 cm s^{-1}. As a measure of acoustic orientation we calculated the cricket's overall lateral deviation towards the left or right side from a forward path (Fig. 2a; middle), and as a measure of steering we calculated the actual lateral steering velocity by which the animals steered to the left and right side (Fig. 2a; bottom). All females tested ($N = 10$) deviated towards the speaker presenting the four sound pulses. On the basis of previous models of pattern recognition we might conclude that the animals oriented towards the 'better' sound pattern, comprising four pulses[9,10]. The concomitant lateral steering velocity, however, oscillated around zero and was directed both to the left and to the right side (Fig. 2a; lower trace). Conspicuous peaks in the lateral steering velocity were closely linked to the occurrence of sound pulses (Fig. 2b) so that each pair of sound pulses elicited a rapid steering transient with maximum peak velocities of 5 cm s^{-1} towards the side of the active speaker (Fig. 2b; lower trace). Plotting the lateral deviation of the animal at high resolution showed that each sound-evoked velocity-transient led to a corresponding deviation of the animal's path by about 1 mm towards the left or right side respectively. Therefore, overall the animal oriented towards the speaker presenting four pulses (Fig. 2b; middle). We quantified the steering behaviour and averaged the lateral deviation velocity signal over 400 chirps (Fig. 2c). Sound pulses presented from the left elicited a transient velocity peak directed to the left, and pairs of sound pulses from the right caused a transient lateral velocity component to the right. These steering responses occurred with a latency of only 55–60 ms and started during the second sound pulse of a pair.

To determine whether walking or flying crickets will also steer towards individual sound pulses we took the split-song model to its extreme and presented every other sound pulse from opposite directions. Averaging the lateral steering velocity of walking crickets ($N = 15$) clearly showed that the crickets rapidly turned towards the sound pulses presented from the left and right side in alternation (Fig. 2d). Auditory steering responses occurred again after 55–60 ms and lasted for 42 ms, the duration of a pulse period. These reflex-like steering responses had very similar amplitudes and because they were directed in opposite directions they cancelled out and, as in crickets walking under open-loop conditions[26], resulted in a net walking direction midway between both speakers. Crickets not only walk but also fly towards singing males, steering with lateral movements of their abdomen[21]. We therefore tested the flight steering of the same crickets that had walked on the trackball. Females ($N = 6$) were exposed to a constant wind stream to elicit flying and their abdominal steering movements were measured with an opto-electronic camera[27] (see Methods). Upon acoustic stimulation the females produced rapid abdominal movements towards the side presenting the sound pattern[28]. Averaging the responses towards the split-song model revealed that they followed the same time course as the steering responses during phonotactic walking (Fig. 2e). These results imply that cricket auditory orientation during walking and flight is based on rapid reactive steering towards individual pulses of the male's calling song.

On the basis of this finding we expected a clear relationship between the number of sound pulses perceived from each side and the overall lateral deviation in walking crickets. We therefore systematically varied the ratio of sound pulses presented from the left or the right. Chirps with six pulses were used and one, two or

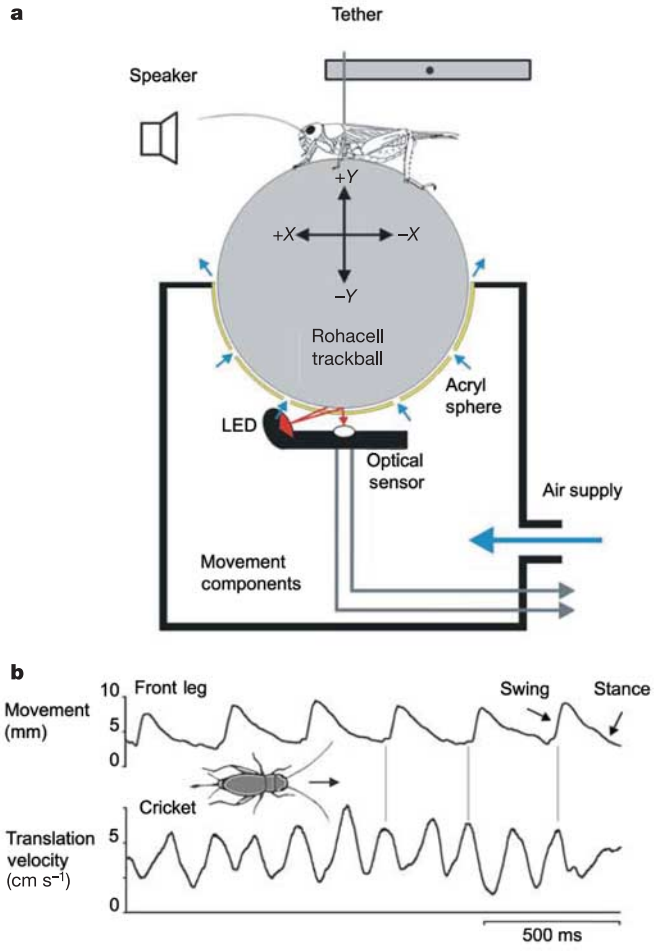

Figure 1 Trackball system for testing auditory orientation of crickets. **a**, A cricket is tethered on a trackball that is floating in an air stream. An optical sensor picks up the movements of the trackball, which is rotated by the cricket walking. **b**, Simultaneous recording of the stance–swing movements of one front leg femur (top, see Methods) and the translation velocity of the cricket (bottom). Owing to insects' tripod gait the translation velocity oscillated with twice the frequency of the front leg movements. Every second velocity peak is in phase with the swing phase of the front leg.

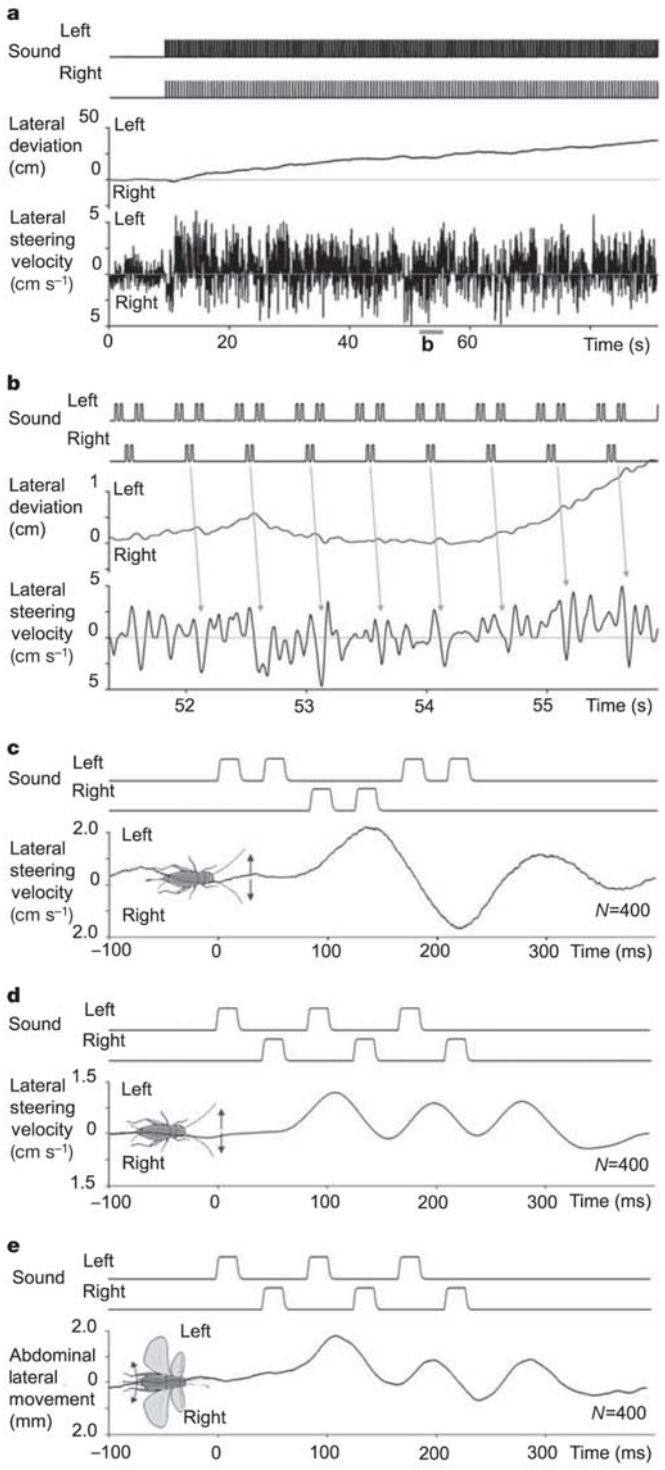

Figure 2 Cricket orientation to songs that are split between two speakers. **a**, The cricket walked towards the left speaker, which presented double the number of pulses. The lateral steering velocity oscillated around zero revealing steering transients to the left and right side. **b**, A high-resolution plot of the section labelled 'b' in **a** shows that each pair of sound pulses elicited a steering transient towards the corresponding speaker. **c**, Dynamics of rapid steering revealed by averaging the lateral steering velocity for 400 chirps. Each pair of sound pulses elicited a steering transient towards the side presenting the sound. **d, e,** Rapid steering in walking and flying crickets. Consecutive sound pulses were presented from alternating sides. The lateral steering velocity of the walking cricket (**d**) and the abdominal steering response of the same cricket during flight (**e**) demonstrate steering towards the sound pulses with the same temporal dynamic during walking and flight.

three pulses were randomly presented from the opposite direction (Fig. 3a; top). When all six sound pulses were presented from one side the animals oriented towards that speaker and the lateral steering velocity components were directed to that side (Fig. 3a; middle). As the number of sound pulses presented from the opposite side was increased, the animals deviated less to the side presenting the larger number of sound pulses. When both speakers presented an equal number of pulses the crickets walked forward and the lateral deviation was close to zero. Although the lateral deviation decreased, the females continued walking and covered an overall distance of 200–230 cm in 30 s in all tests (Fig. 3b). We quantified the overall lateral deviation as a function of the ratio of sound pulses presented (Fig. 3c). All females ($N = 11$) oriented towards the side presenting the larger number of sound pulses. With all sound pulses coming from one side they reached a control value of 100%. Because individual sound pulses elicited similar steering responses (Fig. 2d) we were able to calculate the expected performance of the animals and compare it with what we observed. At a ratio of 5:1 the females steered towards the speaker presenting five pulses with 68.9% (±2.5 s.e.m.) of the reference value (expected 66.6%), at a ratio of 4:2 the deviation was 38.8% (±3.2 s.e.m.) of the reference value (expected 33.3%) and at a ratio of 3:3 it was 6.0% (±2.4 s.e.m.) with zero deviation expected. When exposed to two sound sources the animal's course depends on the ratio of pulses perceived from both sides and emerges as a result of numerous consecutive steering events towards individual sound pulses.

Consequently, we expected crickets to walk straight ahead when exposed to split-song patterns that contain identical numbers of sound pulses but when presented alone have a different attractive-

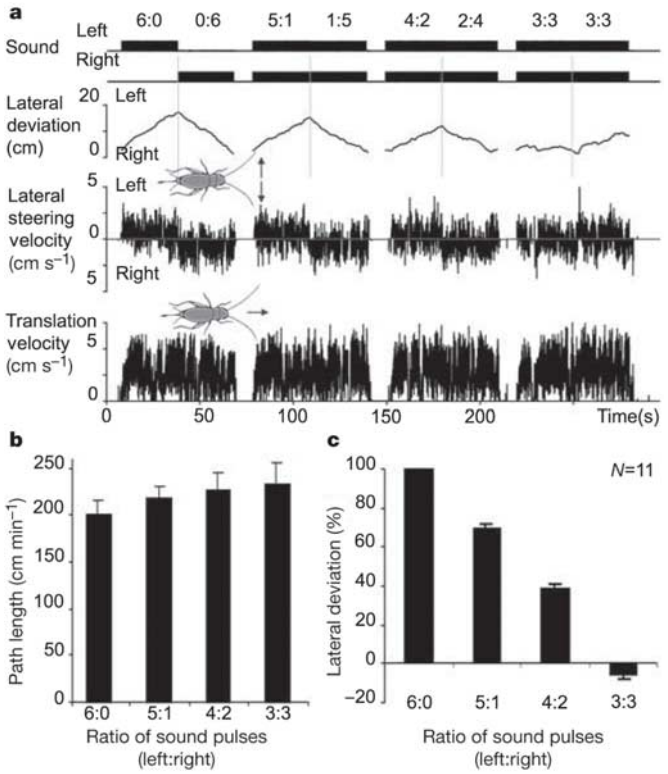

Figure 3 Cricket orientation to randomly split songs with different numbers of pulses presented from each side. **a**, The cricket deviated towards the side with more sound pulses per chirp; however, the animal produced steering transients directed to the left and right side. **b**, Path length walked, according to the ratio of sound pulses presented from opposite directions. **c**, The relative lateral deviation to the side presenting the larger number of sound pulses depends on the ratio of presented sound pulses. Error bars indicate s.e.m.

ness. A sound pattern of three pulses spaced with an interval of 147 ms is only weakly attractive and reached 14.0% (±4.6) of the reference value (Fig. 4a, b; left). When the interval between the pulses was 21 ms, crickets clearly oriented with the reference response of 100% (Fig. 4a, b; middle). Steering towards the 21-ms-interval pattern, however, was reduced to 23.7% (±4.8) when both patterns were presented simultaneously (Fig. 4a, b; right). An average of the lateral steering velocity shows that the animals consecutively steered towards the sound pulses of both patterns (responses to the left indicated by asterisks in Fig. 4c) with the initial response towards the first pulse of the 147-ms-pattern (Fig. 4c). Thus, orientation was not determined by the "better" pattern[10], but by the number of sound pulses presented to each side. The result also indicates that the steering response towards the non-attractive pattern may have been altered when it was combined with the attractive pattern.

The phonotactic behaviour of female *G. bimaculatus* depends on the temporal structure of the sound pattern[4,17,18]. However, when exposed to songs with the species-specific pulse duration and pulse interval, females rapidly steer towards individual sound pulses. This steering behaviour does not support current models for the interaction of pattern recognition and localization. It was proposed that the animals compare the outputs of two separate pattern recognizers on either side of the brain to calculate a steering direction for phonotactic walking[8,9] and concluded that cricket auditory localization is governed by the rule 'turn to the better pattern'[10]. Our results contradict this model because orientation emerges from rapid steering towards individual sound pulses and has already been initiated before the central nervous system has had time to process the second pulse of a chirp. For the same reason, our results rule out the possibility that pattern recognition by band-pass filtering brain neurons[6], template matching[5] or cross-correlation analysis[7] is directly involved in rapid steering. Any of these processes based on the integration of the temporal pattern would require at least two consecutive sound pulses to determine the temporal structure of the perceived song and thus would be too slow to evoke the observed rapid steering responses. This does not exclude the possibility that the recognition process modulates the rapid steering responses over a longer time course.

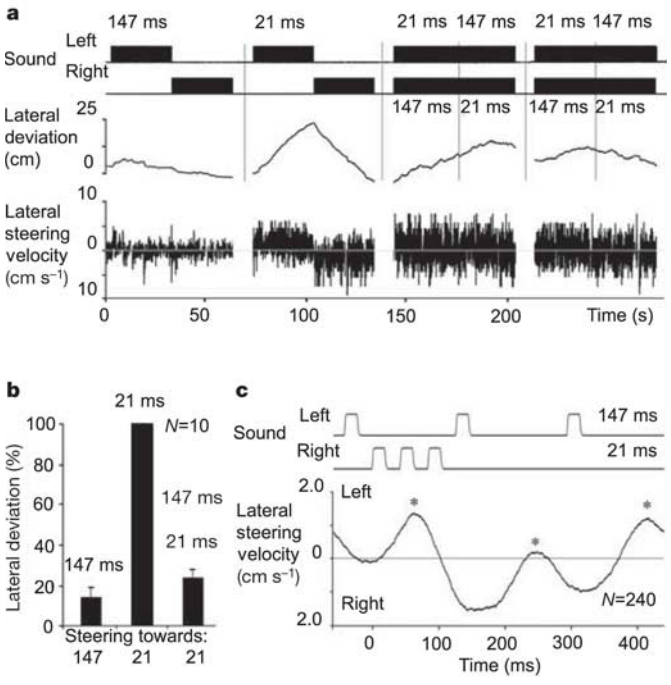

Figure 4 Cricket orientation to patterns of different attractiveness. **a**, A pattern with three sound pulses with intervals of 147 ms elicited only weak orientation towards the sound (first trace). Crickets clearly steered towards a pattern of three pulses with intervals of 21 ms (second trace). Steering towards the 21-ms-interval pattern strongly decreased when both patterns were presented simultaneously (third and fourth traces). **b**, Lateral deviation of ten females to the 147-ms-interval pattern, 21-ms-interval pattern and to the 21-ms-interval pattern when both patterns were presented simultaneously. **c**, An average of the lateral steering velocity ($N = 240$ chirps) shows that the animals steered towards sound pulses from both sides. Error bars in **b** indicate s.e.m.

Cricket auditory orientation emerges from reflex-like reactive steering towards individual sound pulses, and the path of the animals depends on the ratio of pulses perceived from the left and right side. The similarity of the steering responses in walking and flight indicates that the animals use the same strategy and pathway during both behaviours. Using reactive steering towards individual sound pulses, the animals elegantly solve a complex task of auditory orientation with a simple mechanism. This suggests a new concept for cricket phonotactic localization behaviour, based on reactive steering responses to sound pulses. Neuronal activity representing the sound pulses may directly act on the motor control networks[29]. Low level sensory processing with a direct coupling of the sensory input to the motor output has been used in robots and produced cricket-like auditory behaviour[11,12]. Our demonstration of reactive steering[13] in cricket behaviour provides crucial information for the design of neuronal networks driving biologically inspired robots. □

Methods

Crickets

Female crickets (*G. bimaculatus* de Geer) were taken as last instars from a colony at the Cambridge Department of Zoology and were raised individually to maintain phonotactic responsiveness[30]. After the final moult, a metal pin (32 mg; cricket, 1.2 g) was attached vertically with wax to the first abdominal tergites, close to the animal's centre of gravity. The cerci were covered with wax to prevent the animals responding to air currents. The crickets were first positioned in natural walking posture on the top of the trackball and then the attached pins were clamped in the needle holder. While the animals rotated the trackball with their legs their body position and orientation remained constant and thus they were always exposed to identical acoustic conditions. This is an advantage over closed-loop experiments in which movements of the animal will change the impact of the sound stimuli. All experiments were performed in the dark at a temperature of 24–28 °C.

Trackball recordings

The trackball (Rohacell, diameter of 56.5 mm, 3.0 g) fitted into an acrylic half-sphere with 24 evenly spaced holes mounted into a cylinder (Fig. 1a). Air was passed through the holes of the half-sphere so that the trackball was gently lifted and was free to rotate with minimal friction (Fig. 1a). An optical sensor (Agilent ADNS-2051, 2-D Optical Mouse Sensor) was aligned opposite the south pole of the trackball. Looking through the transparent acrylic sphere it monitored any movements of the trackball in the forward–backward (X) and lateral left–right (Y) direction on two separate data channels. Any trackball movement of 127 μm along a measuring axis produced a short (150 μs) coding pulse in the corresponding data channel, with the sign of the pulse coding the movement direction. The system performed linearly up to speeds of 38 cm s^{-1} in either direction. We did not bin the coding pulses, to maintain the maximum sensitivity of the system. The coding pulses for both movement components and the envelope of the sound stimuli were sampled online at 10 kHz per channel using an A/D board (National Instruments PCI-Mio 16-E-4) controlled by software programmed in LabView 5.01. Data were stored on the hard disk of a PC for off-line analysis. From the pulses coding the lateral movements of the trackball, we calculated the actual lateral steering velocity by which an animal steered to the left or right and the overall lateral deviation of the animal from a straight path. From both movement components we determined the translation velocity of the animal and the path length covered in any test series.

Acoustic stimulation

Sound stimuli were generated with Cool Edit 2000 and were presented by standard PC audio boards through two active speakers (Sony SRS A57). These were positioned in front of the cricket at a distance of 66 cm and at an angle of 45° to the left and right of the animal's longitudinal axis. Sound intensities were adjusted to 75 dB SPL relative to 10^{-5} N m^{-2} at the position of the cricket and were measured with a Bruel and Kjaer free field microphone (type 4191) and measuring amplifier (type 2610). The standard sound pattern had a frequency of 4.8 kHz, a pulse duration of 21 ms, an interval of 21 ms, six pulses per chirp corresponding to a chirp duration of 250 ms and a chirp period of 500 ms. To analyse phonotactic walking, each sound pattern was generally presented from the left and right speaker for a minimum of 30 s duration. The envelope of the sound pattern was calculated on-line using an RMS (root mean square) chip (Analogue Devices, type 637) set to a time constant of 0.5 ms. The experiments were performed inside a sound-proofed chamber. The noise level measured at the top of the trackball was 38 dB SPL (band-pass filter 200–200,000 Hz) with the trackball air supply turned on.

Recording of leg movements and flight steering

Stance–swing movements of a front leg during walking (Fig. 1b) or the lateral steering movements of the abdomen[20] during flight (Fig. 2e) were measured with an optoelectronic camera[27]. A piece of reflective disc (3M, Scotchlite 7610) was attached to the femur or abdomen and illuminated. The reflected light was picked up by a position-sensitive photodiode and indicated the position of the body part. For measurements during flight, crickets were tethered by their pins in front of a constant air stream to elicit flying. Conditions of acoustic stimulation were otherwise identical to the trackball experiments.

Received 13 May; accepted 25 June 2004; doi:10.1038/nature02787.

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Acknowledgements We thank our Cambridge and Edinburgh colleagues for comments on the manuscript. The BBSRC and the Royal Society supported the project. We are grateful to M. Knepper and P. Williams for the development of software and hardware and to Röhm GmbH for providing Rohacell.

Competing interests statement The authors declare that they have no competing financial interests.

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MicroRNAs act sequentially and asymmetrically to control chemosensory laterality in the nematode

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Animal microRNAs (miRNAs) are gene regulatory factors that prevent the expression of specific messenger RNA targets by binding to their 3′ untranslated region[1–3]. The *Caenorhabditis elegans lsy-6* miRNA (for lateral symmetry defective) is required for the left/right asymmetric expression of guanyl cyclase (*gcy*) genes in two chemosensory neurons termed ASE left (ASEL) and ASE right (ASER)[4,5]. The asymmetric expression of these putative chemoreceptors in turn correlates with the functional lateralization of the ASE neurons[6]. Here we find that a mutation in the *die-1* zinc-finger transcription factor disrupts both the chemosensory laterality and left/right asymmetric expression of chemoreceptor genes in the ASE neurons. *die-1* controls chemosensory laterality by activating the expression of *lsy-6* specifically in ASEL, but not in ASER, where *die-1* expression is downregulated through two sites in its 3′ untranslated region. These two sites are complementary to *mir-273*, a previously uncharacterized miRNA, whose expression is strongly biased towards ASER. Forced bilateral expression of *mir-273* in ASEL and ASER causes a loss of asymmetric *die-1* expression and ASE laterality. Thus, an inverse distribution of two sequentially acting miRNAs in two bilaterally symmetric neurons controls laterality of the nematode chemosensory system.

Although miRNAs are abundant in animal genomes, the biological contexts and pathways in which miRNAs operate are only beginning to be explored[3]. The *C. elegans lsy-6* miRNA functions in a poorly understood developmental context, the generation of neuronal diversity along the left/right axis of an animal[4,5]. *lsy-6* superimposes a left/right asymmetric expression profile of putative chemosensory receptors, encoded by the *gcy* genes, onto the bilaterally symmetric differentiation program of two chemosensory neurons, ASEL and ASER[4,5]. Asymmetric *gcy* chemoreceptor expression in turn correlates with the left/right asymmetric chemosensory capacities of ASEL and ASER[6]. An essential prerequisite for ASEL/R laterality is the restriction of *lsy-6* expression to the ASEL neuron[5].

To gain a better mechanistic understanding of *lsy-6*-mediated lateralization of the ASE neurons, we conducted genetic screens for mutants that show defects in asymmetric expression of ASE-specific putative *gcy* chemoreceptors (see Supplementary Information)[7]. One of the alleles retrieved from this screen, *ot26*, showed a 100% penetrant *lsy* phenotype in adult animals; both ASE cells expressed the normally ASER-specific *gcy-5* gene and concomitantly lost the expression of the normally ASEL-specific *gcy-7* gene (Fig. 1a, b). The expression of several cell-fate markers that label bilaterally symmetric aspects of ASEL/R differentiation were unaffected (data not shown).

Further analysis of *ot26* mutants showed that the laterality defects at the genetic level were tightly correlated with defects at the behavioural level. Previous studies of chemotaxis behaviour in worms, in which either the left or right ASE neuron was killed,