

Spatio-temporal patterns of antennal movements in the searching cockroach

Jiro Okada* and Yoshihiro Toh

Department of Biology, Graduate School of Sciences, Kyushu University, Fukuoka 812-8581, Japan

*Author for correspondence (e-mail: jokadsch@mbox.nc.kyushu-u.ac.jp)

Accepted 19 July 2004

Summary

To characterize the spatio-temporal patterns of antennal behavior in insects, the voluntary movement of both right and left antennae was examined in the cockroach *Periplaneta americana*. The position of the tip of the antenna (flagellum) is controlled by two mobile joints at its base (the scape and the pedicel) and by the neck. Horizontal and vertical components of movement at the antennal basal joints exhibited rhythmic activities during locomotory (walking) and non-locomotory (pausing) states in the searching animal. In both states, the horizontal component was slower than vertical one. Joint-manipulation experiments suggested that the faster vertical component is due mainly to movements of the scape–pedicel joint, while the slower horizontal component may originate from the head–scape joint. Large horizontal deflections of the antenna corresponded

consistently with the yaw component of head movement. The trajectories of the antennae showed little patterned regularity in most animals. In a few cases, however, loop-like patterns appeared. The area scanned by an antenna was narrower in the walking state than in the pausing state, mainly because of a decrease in the horizontal angular range. Cross-correlation analyses revealed that the coupling between right and left horizontal antennal motor systems and that for the vertical systems were both significantly stronger in the walking state than during pausing. These results indicate that the spatio-temporal pattern of antennal movements changes dynamically depending on the animal's behavioral state.

Key words: insect, cockroach, *Periplaneta americana*, antenna, voluntary movement, searching behavior.

Introduction

To obtain information about the external environment, insects employ a variety of sensory systems. The antenna is the most forward-placed multimodal sensory appendage on the head, and has numerous sensory hairs, each bearing one or a few sensory neurons, a mixture of chemical, mechanical, thermal and humidity receptors (Schneider, 1964; Schafer and Sanchez, 1973; Norris and Chu, 1974; Toh, 1977; Schaller, 1978; Altner and Prillinger, 1980; Chapman, 1982; Keil and Steinbrecht, 1984; Steinbrecht, 1984; Zacharuk, 1985; Lee and Strausfeld, 1990). A striking feature of the insect antenna is that it moves actively to scan the surroundings (Bell, 1991), which may contribute to the efficient collection of information about the immediate environment. Sensory information from the antenna must be essential, especially for insects that are active where light is not available, such as those with nocturnal, underground or cavernicolous lifestyles.

Active movements of insect antennae have been described in relation to visual input (Honegger, 1981; Erber et al., 1993; Horseman et al., 1997; Ye et al., 2003; Lent and Kwon, 2004), olfaction (Suzuki, 1975; Rust et al., 1976; Erber et al., 1993; Lent and Kwon, 2004) and tactile sense (Saager and Gewecke, 1989; Erber et al., 1993, 1997; Pelletier and McLeod, 1994; Ehmer and Gronenberg, 1997a,b; Okada et al., 2002). These reports, however, focused on the antennal response to various

external stimuli. Basic antennal behavior, in terms of the spontaneous rhythmic activity itself, has been described in little detail. Moreover, as most insect antennae are able to move in any direction, their activity has rarely been measured in three dimensions. To our knowledge, the only exception is a study on the stick insect (Dürr et al., 2001), in which voluntary antennal movement during walking was analyzed three-dimensionally.

Each antenna of the American cockroach *Periplaneta americana* used in the present study consists of two mobile basal segments, the scape and the pedicel, while the remaining long distal group of segment is collectively called the flagellum (Seelinger and Tobin, 1981). The head–scape joint is operated by a muscular system inside the head capsule: a single levator and an antagonistic pair of abductor and adductor muscles, and thus may possess two rotation axes for the horizontal and vertical movements. The scape–pedicel joint is operated by a pair of levator and depressor muscles inside the scape, so can move only vertically. In addition, the neck participates in displacements of the entire antenna. Head movement itself is reducible to yaw, roll and pitch components. Thus, the movement of the antennal tip has six degrees of freedom in total. Vigorous antennal movements are observed during searching behavior, exploratory locomotion for finding

resources. The searching is divisible into two clear phases, the walking and pausing states. In both cases, the pair of antennae actively probes their surroundings.

The purpose of this study was to characterize the spatial and temporal properties of antennal movement in the cockroach. Trajectories described by the flagellum tip were classified into some spatial patterns. Spectral analyses combined with joint manipulations revealed the temporal features and gross kinematics about the antennal joints. Cross-correlation analysis between the paired horizontal and vertical components at the antennal joints was used to clarify that coupling between the right and left antennal motor systems can flexibly change, depending on the animal's behavioral state. Preliminary reports have appeared elsewhere in abstract form (Okada et al., 2001; Okada and Toh, 2002).

Materials and methods

Animals

Adult male cockroaches *Periplaneta americana* L. were hatched and reared in a laboratory culture, kept at 27°C, and fed rodent pellets. Before experiments, animals were immobilized on ice, and both the compound eyes and the ocelli were cauterized with current-heated thin Nichrome wire (0.2 mm diameter) in order to eliminate all vision. This process for depriving vision was important for initiation and continuation of the searching behavior. If the operation were not completed, the animal would tend to run when exposed to room lighting. A thin flexible plastic plate (0.5 cm×3 cm) was glued to the pronotum and attached to a clamp stand, to restrain the animal. To assist in registering head movements, several white spots were painted at appropriate positions on the head capsule. Experiments on treated animals were initiated at least 2 days later, to allow for recovery from the operation.

Experimental apparatus

A tethered animal was placed on top of a treadmill formed from a free-moving Styrofoam ball to allow walking (Fig. 1) as described in our previous report (Okada and Toh, 2000). Movements of the antennae and head were recorded simultaneously by a set of three video cameras (30 Hz frame rate), positioned directly above, in front and to one side of the animal's head. Images from the cameras were processed by a video multi-viewer (MV-40E, For-A Co., Tokyo, Japan) to synthesize them on a four-way split screen, and videotaped. The data were replayed and stored on a PC as AVI-formatted files through a video-capturing interface, and then translated to BMP-formatted sequential still images (640×480 pixels) using video-editing software (Adobe Premiere; Adobe Systems Inc., San Jose, CA, USA). Spatial resolution of each video frame was at 125–188 µm/pixel. To register the coordinates of body parts, the still images were sequentially imported to a custom program written in Microsoft Visual Basic (Microsoft Co., Redmond, WA, USA). The positions of the appendages and head (see the following section for detail) were manually plotted frame-by-frame (33.3 ms interval) to register their two-

dimensional coordinates on the PC screen. The data were saved as Text-formatted files.

Data analysis

To measure the positions of the head and both antennae, the body and head axes were defined as reference (Fig. 2). The body axis is identical with the animal's midline from the top view, and from the lateral view it passes the center of the neck socket and runs parallel to the ground. The head axis is identical with the rostrocaudal axis of the head capsule from the top view, and perpendicular to the major flattened part of the frons from the lateral view (Fig. 2A). The intersection of the body and head axes, always lying near the center of head, was defined as the origin for measuring subsequent angular displacements of the head and the both antennae.

Head movements were categorized into three rotations: the yaw, roll and pitch components. We focus here on the two prominent yaw and pitch components. The yaw was defined as deviation of the head axis from the body axis from the top view (Fig. 2Ai), and the pitch as that from the lateral view (Fig. 2Aii). The roll was defined as rotation of the dorsoventral axis of the head capsule from the perpendicular (Fig. 2Bii). Because spatial resolution of images was noisy around the head capsule, time-series of these head positions were smoothed with a sliding window width of three frames (100 ms) to reduce effects of errors due to the manual digitizing. This process may not affect the head components since their frequencies were usually at less than 1 Hz in the present experimental condition. In the following, the yaw and pitch components are also referred to as the horizontal and vertical components, respectively. In both the locomotory and non-locomotory searching, the maximum displacement in yaw and pitch often attained a 35–40° deflection. By contrast, roll was more restricted, 15° at most. The head capsule itself may also translate without rotation, but its effect on the orientation of the antennal tip was thought to be quite small compared to the effects of head rotations. Thus, we took no account of such translatory head movements in this study.

The horizontal and vertical deflections in antennae were defined as, respectively, azimuth and latitude of the flagellum with respect to the head axis (Fig. 2Bi). Points for registering antennal position were set at about a half-length of the flagellum on the PC screen. For horizontal deflections, to the right to the head axis was defined as positive, to the left as negative. For vertical deflections, the upper side to the head axis was defined as positive, the lower side as negative. Transformation of antennal positions from Cartesian to polar coordinates was performed in Microsoft Excel. The horizontal angular position ranged usually from –30° to 120° for the right antenna and the opposite for the left one (–120° to 30°). But occasionally, it jumped suddenly to extreme positions with large displacements (>100°/frame). This unusual antennal shift was undoubtedly an artifact coming from measurement error in which discrimination was insufficient between antennal and head components. Such errors mostly occurred in the prominent dorsoventral deflections of both the antenna and the

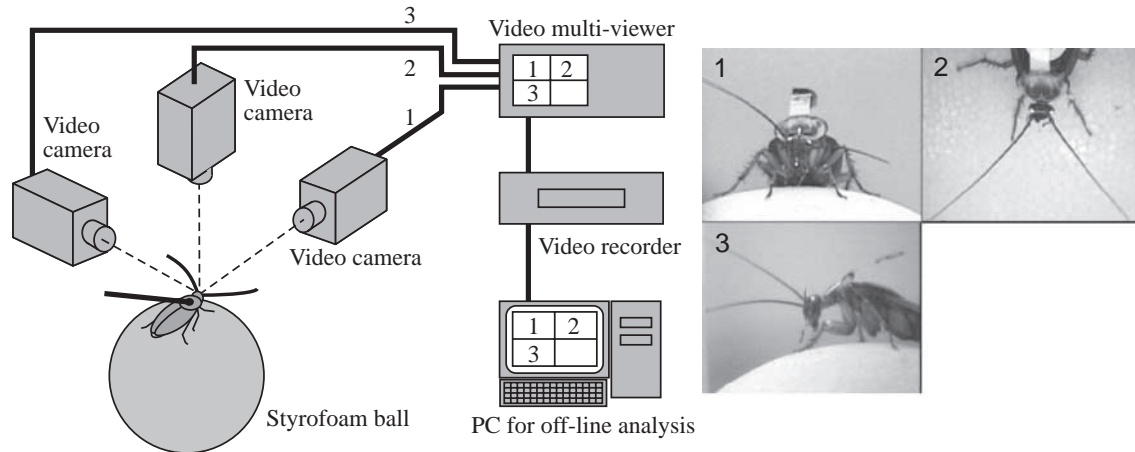


Fig. 1. Experimental apparatus for recording antennal movements. An animal was tethered on a free-moving Styrofoam ball to allow its unrestrained locomotion (Okada and Toh, 2000). Three video cameras were set just in front (1), top (2) and side (3) of the animal. Video images were processed by a 4-split multi-viewer, videotaped, and analyzed using a PC. Photograph on the right is an example of synthesized video frame after the processing, showing frontal (1), top (2) and side (3) views.

head. Since the movements had serious effects on the spectral and cross-correlation analyses that were difficult to treat, we neglected these occasional shifts in the analyses.

Movements of the foreleg were measured as horizontal angular displacements of the tarsus from the body axis (Fig. 2Ai). The origin was set at the posterior end of the pronotum on the body axis. For the foreleg position, to the right to the body axis was defined as positive, and to the left as negative.

Spectral analyses on the rhythmic antennal movements were performed by using the fast Fourier transformation (FFT)

function of Origin (OriginLab Co., Northampton, MA, USA). Horizontal or vertical antennal positions for >30 s were sampled, and processed by the FFT function. Cross-correlation analyses for exploring couplings between a pair of time-series were also conducted using Origin. Temporal sequences of antennal movements (23–48 s samples) were partitioned sequentially into blocks of 3 s (90 data points), and correlation coefficients (r) were calculated for each block over a range of ± 1 s (lags of ≥ 30 data points). The critical value was determined as $r=0.25$, according to the number of data points used for the calculation in each test block ($N=60$). In order to

compare coupling strengths between the locomotory and nonlocomotory states quantitatively, the correlation index (CI) was employed, and defined as the average of the correlation coefficients at zero time lag for a series of blocks.

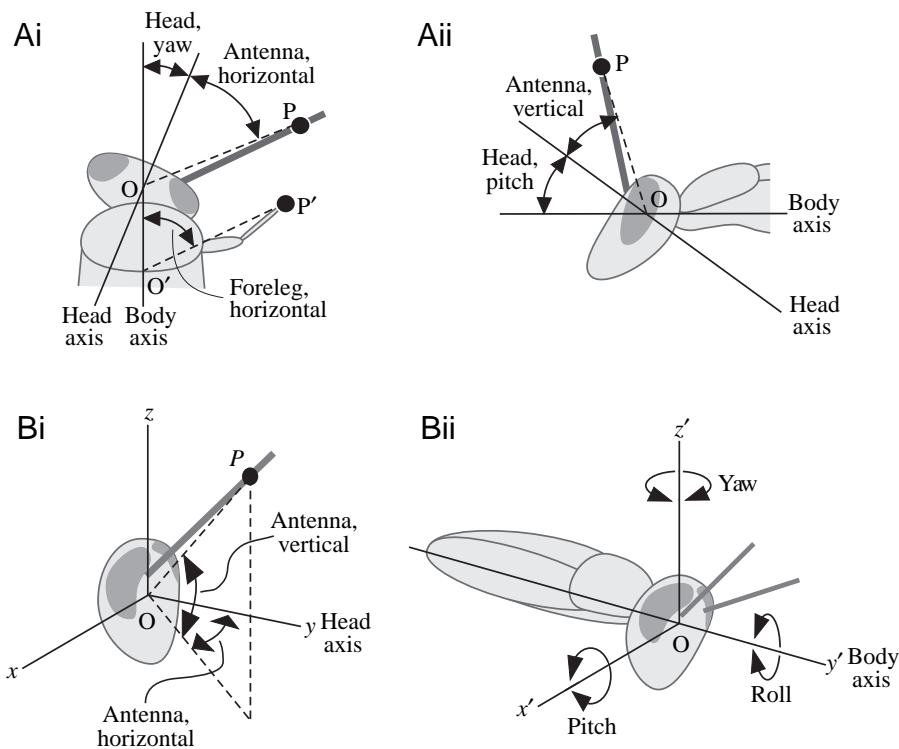


Fig. 2. Method for measuring positions of the antenna, head and foreleg. (A) Top view (i) depicts horizontal angular positions of the right antenna and the foreleg, and yaw position of the head. Lateral view (ii) is a diagram for the antennal vertical and head pitch positions. (B) Relationships of parameters for the antennal (i) and head (ii) positions. Note that three-dimensional coordinates for antennal (i) and head (ii) positions are different from each other. The rostrocaudal y axis for the former is identical with the head axis, and the latter (y' axis) with the body axis. See Materials and methods for details of these parameters. O, origin for both antennal and head positions; O', origin for foreleg position; P, plotting point registering antennal position; P', plotting point registering foreleg position.

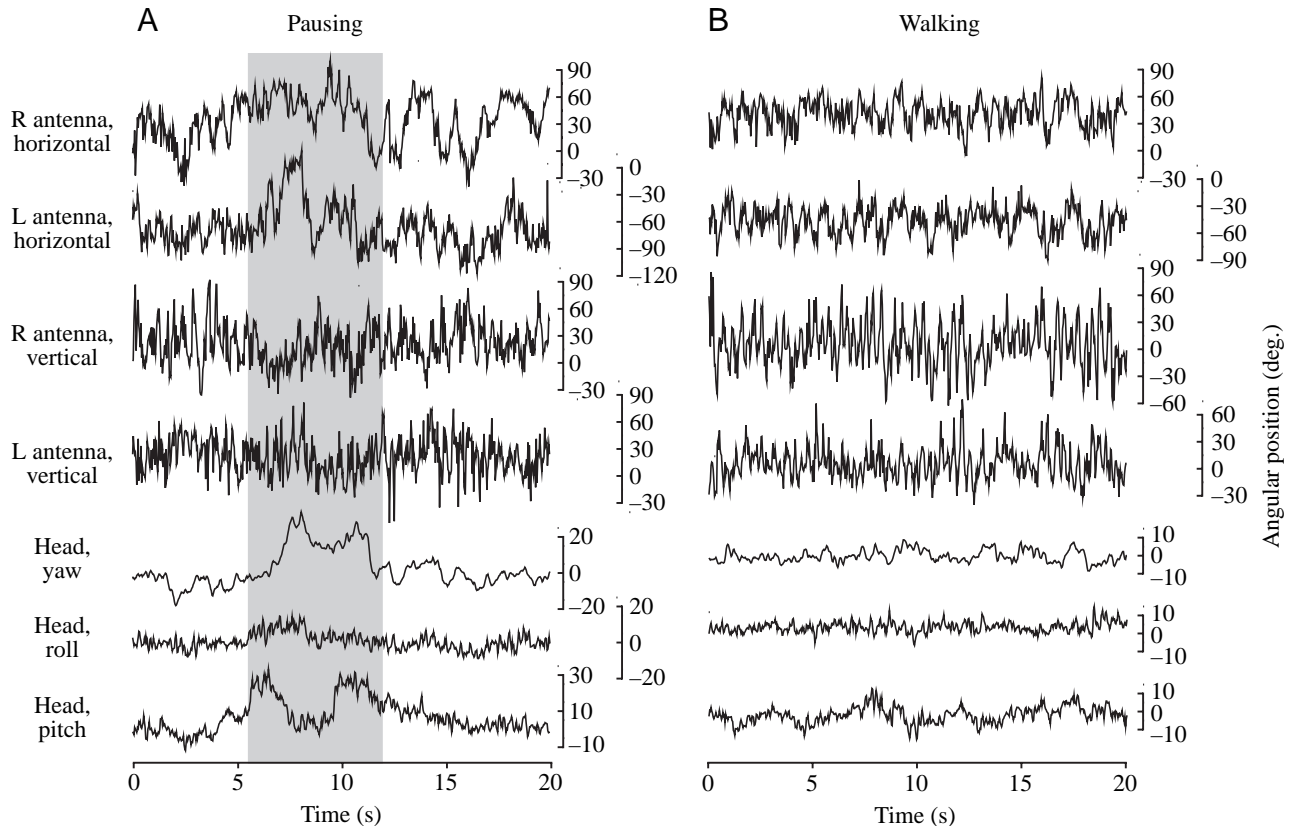


Fig. 3. Antennal and head movements during pausing (A) and walking (B) in a searching cockroach. These two examples were obtained from the same animal. In pausing, large deflections were frequently observed in traces of the antennal horizontal components and the yaw and pitch components of the head (see shaded area). Antennal vertical arcs were lowered entirely in the walking state. Deflections in the head roll were relatively small in both behavioral states. Vertical calibration bars show angular positions for antennae and head. L, left; R, right.

Differences between two data groups were examined statistically by the Wilcoxon test or the Mann–Whitney *U*-test. A result was judged significant when $P < 0.05$.

Results

Searching behavior on the treadmill

When animals were mounted on the treadmill, they often ran at first, but gradually slowed their speed down over a few minutes, and finally started searching with vigorous antennal movements and frequent zigzag turns, as observed in normal free-walking behavior. This searching behavior on the treadmill could continue for a few minutes up to more than an hour. The behavior was clearly distinguishable into pausing and walking. The duration of the pauses varied: short-term pauses for a few seconds were very frequent, occurring several times per minute, whereas long-term pauses (>10 s) were rare. To compare antennal movements between the two behavioral states, large data sets were necessary. We therefore selected ten examples of appropriate length (23.3–48.0 s) as representative pausing state data from different animals, and then sampled the same length data for the walking state from each of the same animals.

Spatio-temporal aspects of the antennal and head movements

Active movements of both the antenna and the head were observed regardless of the searching mode (Fig. 3). This indicates that there is no substantial difference in both the antennal and head movements between pausing and walking. However, as their time courses were compared carefully, some quantitative differences emerged (see the following).

The waveforms of the horizontal components resembled each other for the right and left antennae, suggesting that large amplitude deflections occur simultaneously in both antennae (Fig. 3). The relationship in the pair was anti-phase, i.e. an outward movement (abduction) in one antenna accompanied by an inward movement (adduction) of the other, and *vice versa* for movement in the opposite direction. This relationship was surveyed by cross-correlation analyses (see below). Large horizontal deflections during walking often accompanied whole body turns (data not shown).

Fig. 4 shows the angular ranges and the central positions of antennal deflections in pausing and walking. The angular range is given by the maximum and minimum values for the horizontal or vertical component in each test, and consequently, the working range is given as a difference between the two extreme values. The central position is defined

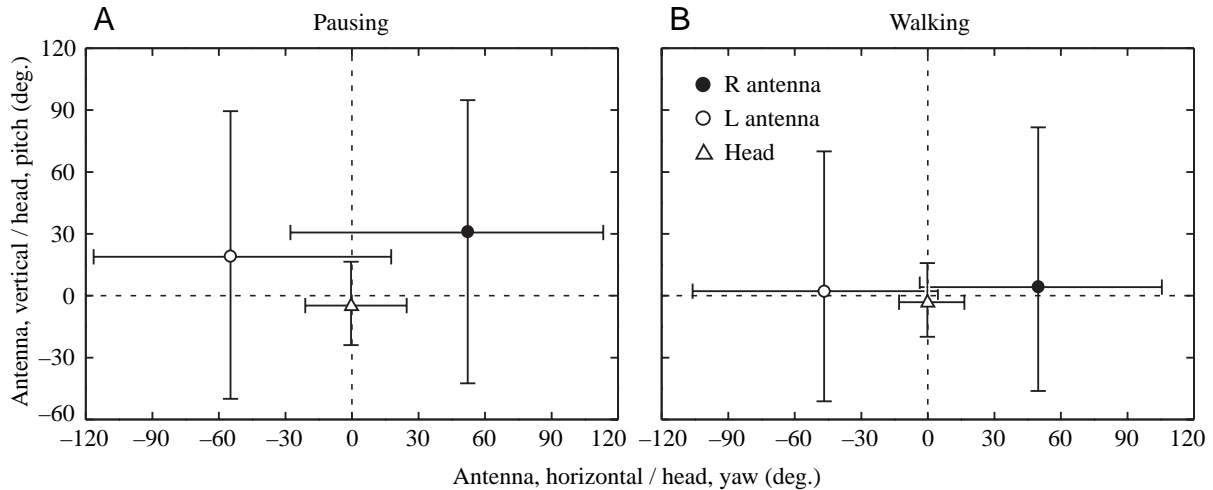


Fig. 4. Angular ranges and central positions for the horizontal and vertical components in both antennae and head. Bars and symbols indicate the mean angular ranges and central positions, respectively ($N=10$). Working ranges for antennal horizontal and head yaw components decreased, respectively, by about $25\text{--}30^\circ$ and 15° during walking, but were not largely changed for antennal vertical and head pitch components. Vertical central positions for both antennae were lowered by $15\text{--}25^\circ$ during walking. R, right; L, left.

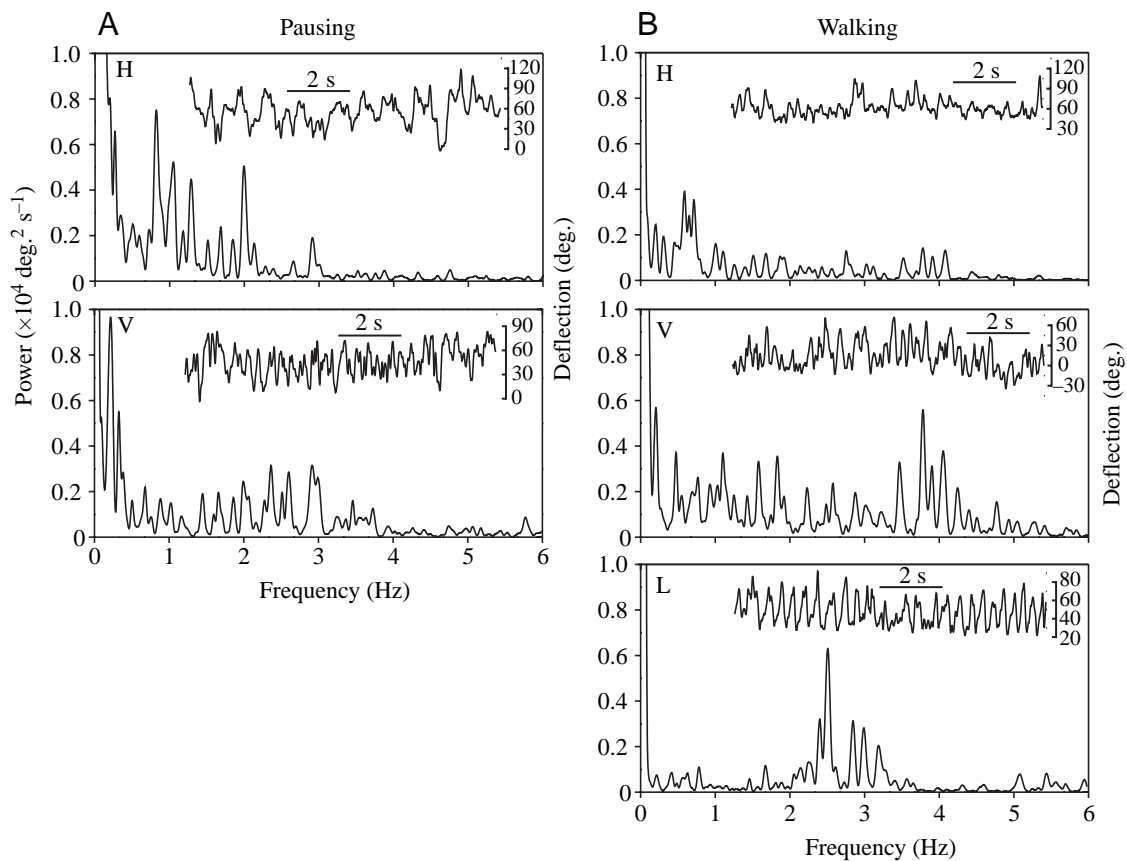


Fig. 5. Power-spectra for antennal and leg movements in a searching cockroach. (A) Spectra for horizontal (H) and vertical (V) deflections in pausing. Their major peaks were distributed mainly at <3 Hz (H) and <4 Hz (V), respectively. (B) Power-spectra for antennal (H and V) and foreleg (L) movements in walking. Lower frequency components at <2 Hz were largely suppressed for horizontal deflections (H). Higher frequency components around 4 Hz became remarkable for vertical deflections (V) instead. Note that no conspicuous peak is observable in the H and V spectra at the frequency corresponding to major peaks in the L spectrum ($2\text{--}3.5$ Hz). Inset in each spectrum is part of the time course before FFT. These spectra were obtained from data for a 31 s period each.

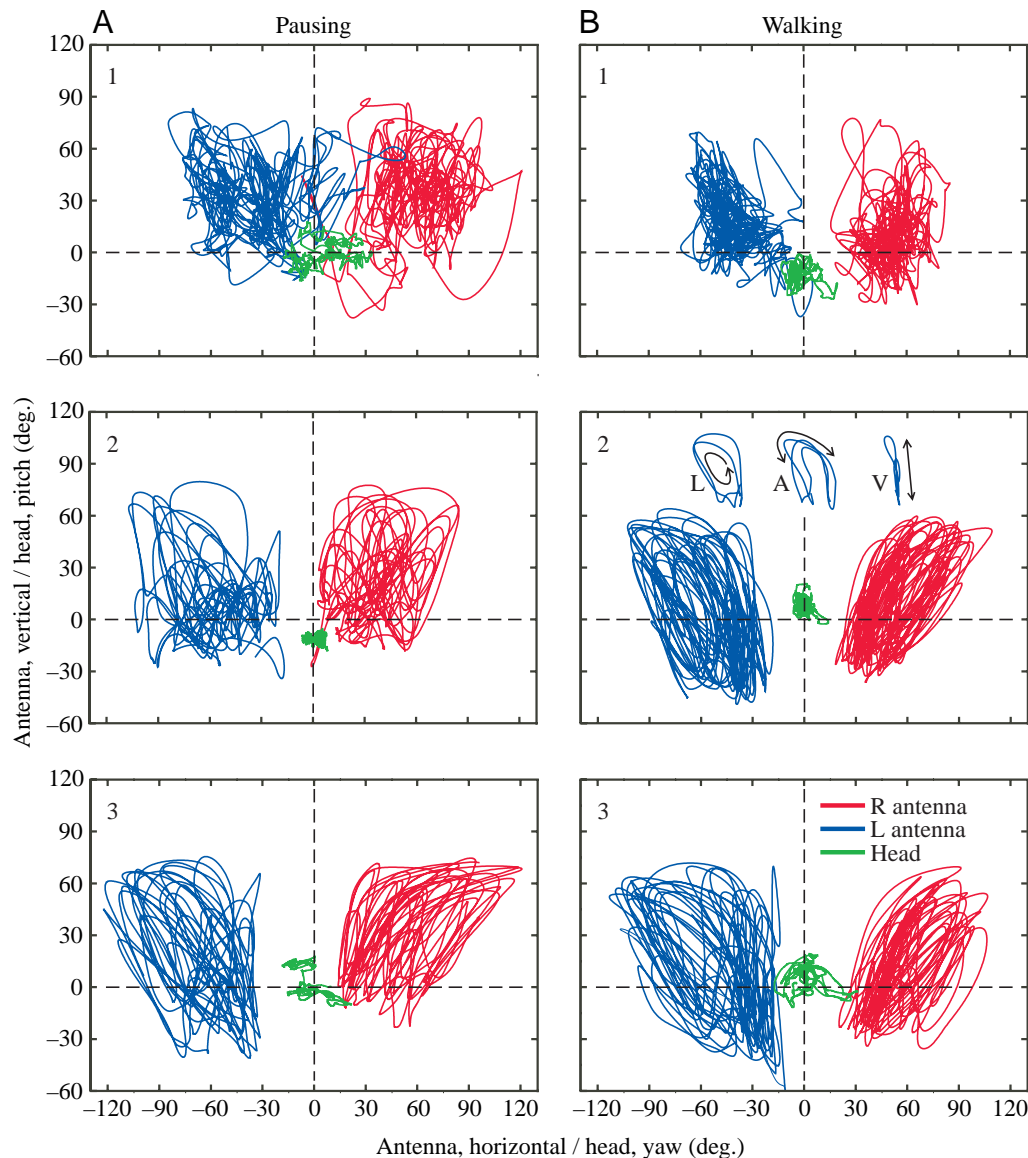


Fig. 6. Trajectories of both antennae and head. Data in cases 1–3 were obtained from three different animals. The sampling period for each graph was 15 s. The right and left antennal trajectories are indicated, respectively, by red and blue traces, and the head by green traces. (1) The random pattern was most commonly observed in both pausing (A) and walking (B). (2) The loop-like pattern was apparent only during walking. Insets in the right panel show typical examples of the three basic patterns forming the loop-like trajectory. These were derived from a trajectory of the left antenna. L, loop; A, arch; V, vertical line. (3) The ‘consistent’ loop-like pattern independent of the searching mode.

as a median in the time-series of the horizontal or vertical component in each test. For the horizontal component, large deflections were observed more frequently in the pausing state than when walking (Figs 3, 4). The mean angular ranges in pausing were from -27.7° to 113.3° , and from -116.4° to 17.7° for the right and left antennae, respectively ($N=10$) (Fig. 4A). By contrast, those in walking were narrowed by 20° to 30° , from -3.6° to 105.5° and from -106.1° to 4.6° for the right and left antennae, respectively ($N=10$) (Fig. 4B). The bilateral overlap formed around the horizontal center was apparently larger in pausing than in walking (45.4° versus 8.2°). When both the right and left antennae were handled together as 20 antennae from 10 animals, there was a highly significant difference in the working range between pausing and walking (Wilcoxon test, $P<0.01$). The mean central positions for horizontal components did not change largely between the two behavioral states. When the horizontal central positions were transformed to the absolute angular deviations from the body axis in order to deal equally with both right and left antennae,

however, they shifted significantly to more medial/frontal positions by about 5° ($P<0.05$).

For the vertical component, the mean angular ranges also changed according to the search mode (Fig. 4); -42.5° to 94.8° (right, pausing) and -50.0° to 89.5° (left, pausing) versus -46.1° to 81.6° (right, walking) and -51.2° to 70.0° (left, walking) ($N=10$). A statistical test detected a significant difference in working ranges between pausing and walking (Wilcoxon test, $P<0.01$). This indicates that the arc of vertical deflections was narrower in walking by 10 – 20° than in pausing. Another change of the vertical component emerged in the mean central position, 30.7° (right, pausing) and 18.9° (left, pausing) versus 4.1° (right, walking) and 2.2° (left, walking) ($N=10$). Both antennae thus point to a lower position in walking ($P<0.01$). In the pausing state, the right antenna tended to be more elevated by 10 – 15° than the left antenna (Fig. 4A). This difference between both antennae was observed in nine animals out of ten, and was highly significant ($P<0.01$). By contrast, there was no such difference in the walking state

(Fig. 4B, $P>0.05$), implying that the tendency in pausing is due to the antennal behavior itself, and not to any artifacts (e.g. incorrect setting of animals to the experimental apparatus). To characterize this biasing in the antennal posture, however, further careful experiments would be required, and this issue remains to be investigated.

The three head components (yaw, roll and pitch) did not exhibit definite rhythmicity in the periods examined (<48.0 s), but fluctuated largely while in the pausing state (Fig. 3). The mean angular ranges for the yaw in pausing and walking were from -21.1° to 24.7° and from -12.9° to 16.4° , respectively ($N=10$) (Fig. 4). The working ranges of the yaw components were significantly narrowed by around 15° during walking (Wilcoxon test, $P<0.05$), but there was no difference in the central position between two behavioral states. For the pitch component, the mean angular ranges in pausing and walking were from -24.0° to 16.5° and from -19.9° to 15.8° , respectively ($N=10$) (Fig. 4), and there was no difference in both the working ranges and the central positions between two states. The yaw deflections consistently corresponded with large horizontal deflections in both antennae, and also with intended body turns (data not shown). It was unclear whether there is some coordination among the three head components or not. In some cases, however, coordinated activities were observed in two or in all three of the head components (e.g. see Fig. 3A, shaded area).

To quantify differences in temporal aspects of antennal rhythmic movements according to the behavioral state, FFTs were performed on the horizontal and vertical components (Fig. 5). In pausing, power-spectra for the horizontal and vertical deflections exhibited their major peaks at <3 Hz and <4 Hz, respectively (Fig. 5A). While walking (Fig. 5B), the horizontal deflection was mostly suppressed at lower frequencies (<2 Hz). This reflects, as described above, a decrease in the large horizontal deflections during walking. For the vertical component, particular peaks additionally appeared around 4 Hz, suggesting that the movement became faster. Actually, the waveforms in walking are composed of faster and larger discrete sinusoids than in the pausing state (Fig. 5, insets, and more clearly in Fig. 3). Though frequency bands differed more-or-less, the same tendencies were observed in the horizontal and vertical components in 16 and 13 examples, respectively, out of 20 antennae from 10 animals. Forelegs moved rhythmically, mainly

at 2–3.5 Hz, during walking (Fig. 5B). Nevertheless, there was no conspicuous peak in the spectra for both horizontal and vertical components at the corresponding frequency band (2–3.5 Hz). It is therefore unlikely that passive effects, for instance caused by inertia, were involved in the antennal movements.

Trajectory patterns of the antennal and head movements

Trajectories of antennal and head movements were depicted

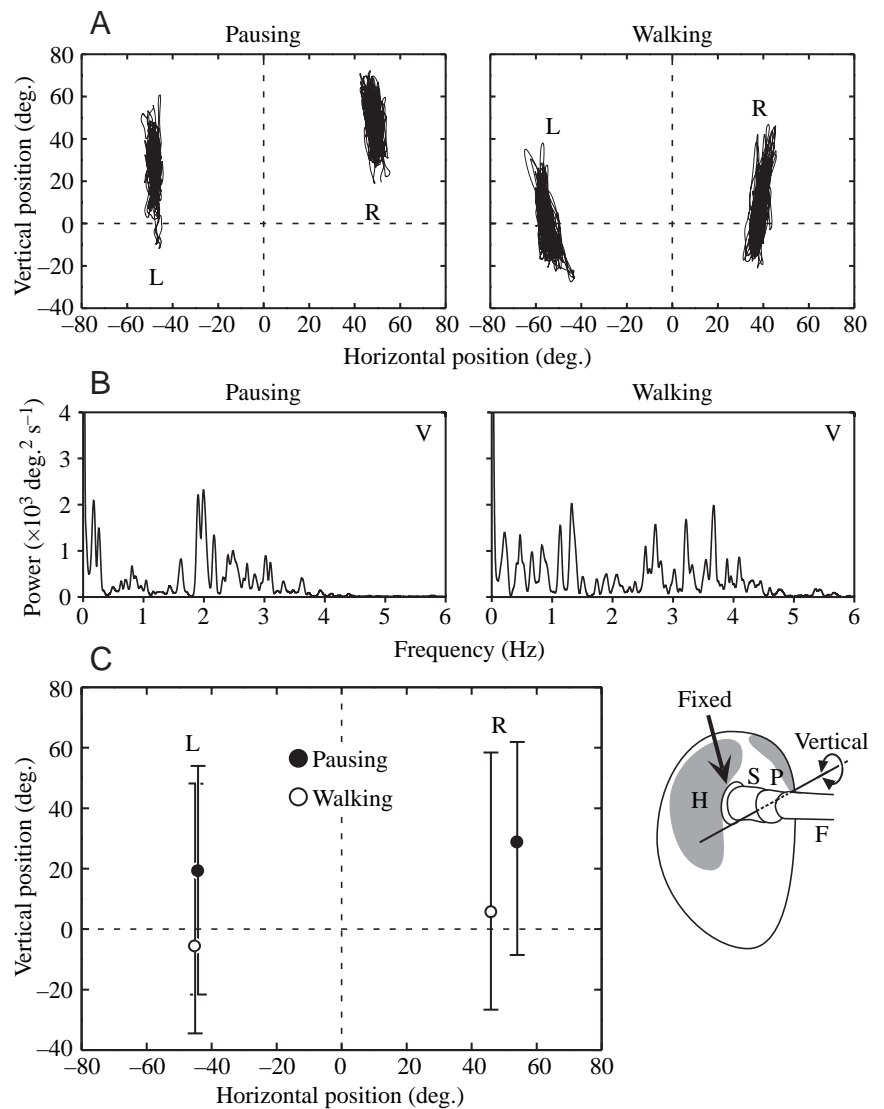


Fig. 7. Kinematics of the scape–pedicel (S–P) joint in two behavioral states. By immobilizing the head–scape (H–S) joints (see inset at bottom), movement of the S–P joint could be analyzed independently. (A) Trajectory patterns formed simple vertical lines in both pausing and walking. Vertical positions were lowered entirely by $20\text{--}30^\circ$ during pausing. (B) Power-spectra for vertical components (V) in the two states. These spectra were obtained from the time-series whose trajectories are shown in A. Discrete peaks appear at 1.5–3.5 Hz during pausing, but distributed over a wider range up to 4.5 Hz in walking. (C) Spatial profiles of the S–P joints in pausing and walking. Bars and symbols indicate averages of the angular ranges and the central positions, respectively ($N=4$). Both antennae significantly lowered by about 25° during walking. Inset shows a rotation axis of the free S–P joint in this condition. Sampling periods were 30 s (trajectories) and 34 s (power-spectra). H, head; S, scape; P, pedicel; F, flagellum.

by two-dimensional coordinates (Fig. 6). The most frequent trajectory of the antenna was a seemingly random pattern, i.e.

no apparent spatial regularity in either the pausing or walking state (Fig. 6, Case 1). This random pattern was observed bilaterally in 8 out of 10 animals. The area scanned by an antenna in a constant period was narrower in the walking state than when pausing, mainly because of a decrease in the horizontal deflection. The head behavior was similar to that of the antenna: the trajectory had little regularity, and the angular range of yaw deflections decreased during walking.

Regularity of antennal trajectories, however, was observed bilaterally in the other two examples illustrated. In the second example (Fig. 6, Case 2), the trajectory was rather random in the pausing state, but changed to a loop-like pattern when the animal started to walk. The third example showed the loop-like pattern independent of the locomotory state (Fig. 6, Case 3). The loop-like trajectory was composed not only of the loop, but also of two other basic pattern elements (see Fig. 6B2). The first pattern was a simple loop consisting of four sequential phases: levation, abduction, depression and adduction. In the second pattern, the antenna described an arch, in which a single cycle was formed from six sequential phases: levation, abduction, depression, and again levation, adduction and then depression. The third pattern was a simple vertical line: dorso-ventral movement around the anterior extreme position.

Kinematics of the antennal joints

The position of the antennal tip is controlled mainly by two basal joints, the head-scape (H-S) and scape-pedicle (S-P) joints. The former is like a Cardan joint with two rotation axes and moveable in both horizontally and vertically, while the latter is a hinge and can move only vertically. In order to characterize the kinematics of these joints independently, and to examine the behavioral state-dependency about each joint movement, we tried to record antennal movements in which either the H-S or S-P joint was immobilized with glue. However, most of the treated animals behaved differently from the normal ones: they were depressed in locomotion with almost no antennal movement, and otherwise continued to walk or run with little antennal movement and zigzag turn. We only collected four examples of relatively normal searching for each behavioral mode in each joint-fixing condition (16 samples from 8 animals in total).

Trajectories in the condition with only the S-P joints free were repeated vertical

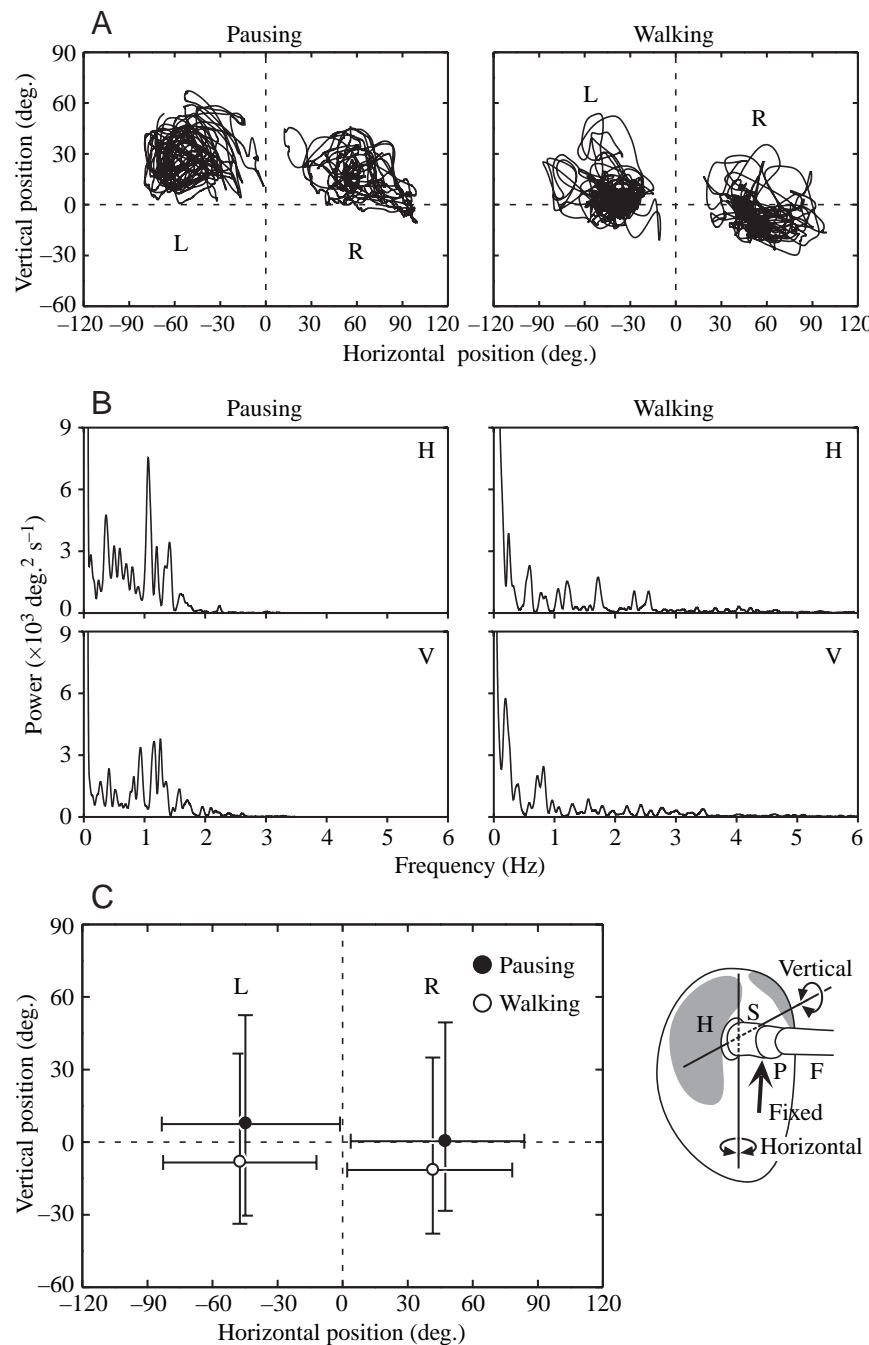
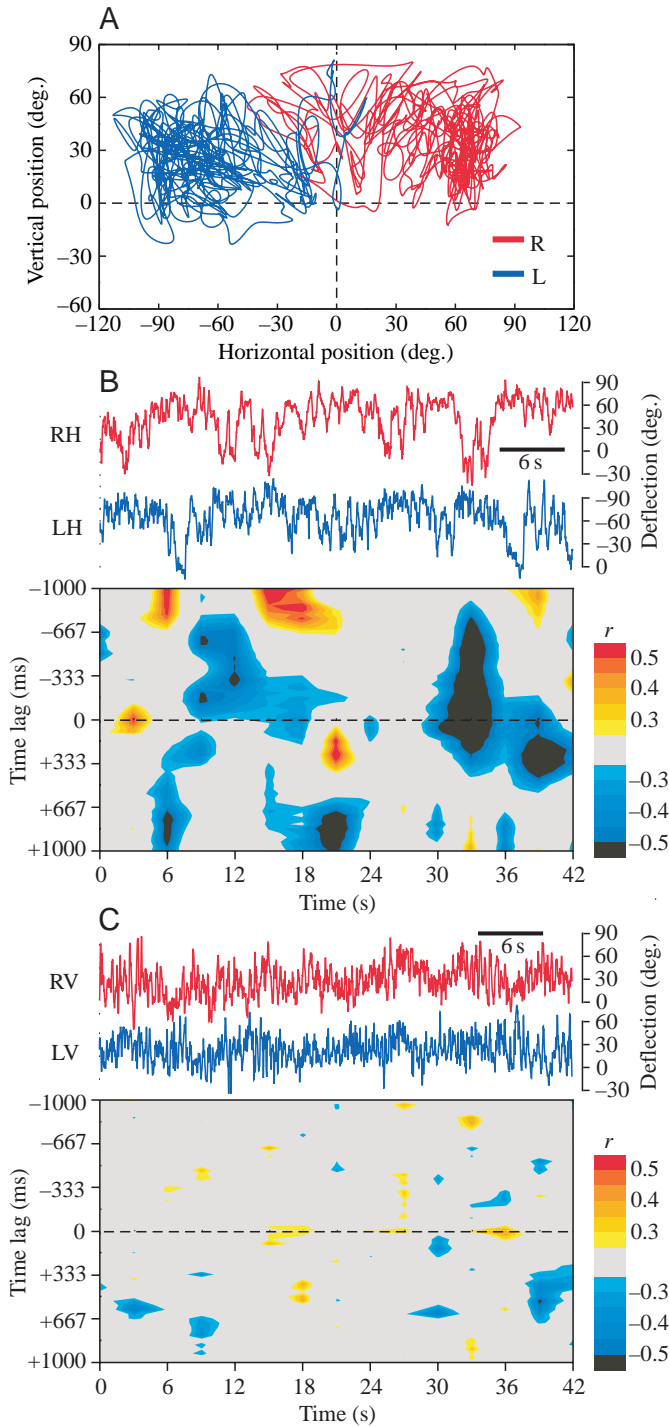


Fig. 8. Kinematics of the head-scape (H-S) joint in two behavioral states. The S-P joint is immobilized in this condition (see inset at bottom). (A) Trajectories showed two-dimensional expansion in both states. During walking, both antennae concentrated at lower positions. (B) Power-spectra of horizontal (H) and vertical (V) components in the two states. Peaks at <2 Hz decreased in walking for both components. These spectra were obtained from the time-series whose trajectories are shown in A. (C) Mean angular ranges (bars) and central positions (symbols) of both antennae during pausing and walking ($N=4$). Vertical central positions for both antennae were significantly lowered as observed in the S-P joint free condition (see Fig. 7). Horizontal working ranges were slightly but significantly narrowed during walking. Inset indicates two rotation axes of the free H-S joint. Sampling periods and abbreviations, see Fig. 7.



excursions in both pausing and walking (Fig. 7A). In the walking state, positions of both antennae were lowered entirely by 20–30°. Fig. 7C shows the mean angular ranges and central positions of the vertical component in both states ($N=4$). The averaged angular ranges in pausing were from -8.6° to 61.9° (right) and from -21.6° to 54.0° (left), while in walking from -26.6° to 58.4° (right) and from -34.5° to 48.2° (left). The averaged central positions in pausing were at 28.4° (right) and 19.4° (left), and those in walking were at 5.5° (right) and -5.6° (left). Considering both right and left antennae together (total

Fig. 9. Cross-correlations between right (R) and left (L) antennal movements during pausing. (A) Trajectories of both antennae show typical random patterns (sampling period 18 s). (B) Cross-correlation for horizontal components. The waves indicate the time courses of the right and left horizontal deflections for a period of 42 s. Note that polarities for the horizontal position are opposite between the right and left antennae (refer to calibration bars). RH, horizontal deflection of the right antenna; LH, horizontal deflection of the left antenna. Correlation coefficients (r) in the correlation map were calculated by reference to the left antenna. Significant positive correlations ($r>0.25$) are indicated by yellow to red, and the negative ones ($r<-0.25$) by blue to black. Significant negative correlations were observed as broad blue or black spots. Backgrounds filled by gray color represent the areas where r values were not statistically significant ($|r|<0.25$). (C) Cross-correlation for vertical components. Significant correlations were few on the map. RV, vertical deflection of the right antenna; LV, vertical deflection of the left antenna.

8 antennae from 4 animals), the working range was significantly wider when walking than that when pausing (Wilcoxon test, $P<0.05$). This result was inconsistent with that in the normal condition (Fig. 4). Considering the sample number analyzed (20 *versus* 8), the data for normal antennae may be more reliable. Alternatively, the behavior of the antenna itself might have changed as a result of the joint manipulation. For the central position, there was a significant difference between the two searching modes ($P<0.05$). Thus, the S–P joint may be involved in searching mode-dependency of the antennal vertical position.

Antennae in the H–S joint-free condition draw two-dimensional patterns that were rather simple compared to those of the normal antennae (Fig. 8A). In particular, the trajectories were formed mostly from circular-pattern units (see Fig. 8A, pausing). The average horizontal angular ranges in pausing were from 3.7° to 83.8° (right) and from -83.3° to -1.2° (left), and in walking from 2.2° to 78.2° (right) and from -82.7° to -12.0° (left) (Fig. 8C). There was a significant difference in the working range between the two behavioral states (Wilcoxon test, $P<0.05$, $N=8$), indicating that the horizontal arcs of antennae were narrower in the walking state. This result was consistent with those for normal antennae (see Fig. 4). The horizontal central position unchanged between the two states ($P>0.05$) in this condition, although it was slightly shifted in the normal antenna to medial/frontal position by about 5° during walking (Fig. 4). For the vertical component, the mean angular ranges in pausing were from -28.4° to 49.5° (right) and from -30.4° to 52.5° (left), and in walking from -37.8° to 35.0° (right) and from -33.7° to 36.7° (left) (Fig. 8C). Though the working range did not alter significantly, the central position was lowered by about 15° ($P<0.05$). The results indicate that the H–S joint also participates in searching mode-dependency of the antennal vertical position as well as the S–P joint.

Spectral analyses applied to antennal movements in joint-manipulated animals revealed their temporal aspects (Figs 7B, 8B). With only the S–P joint free (Fig. 7B), the power-spectrum for the vertical movement had its major peaks at

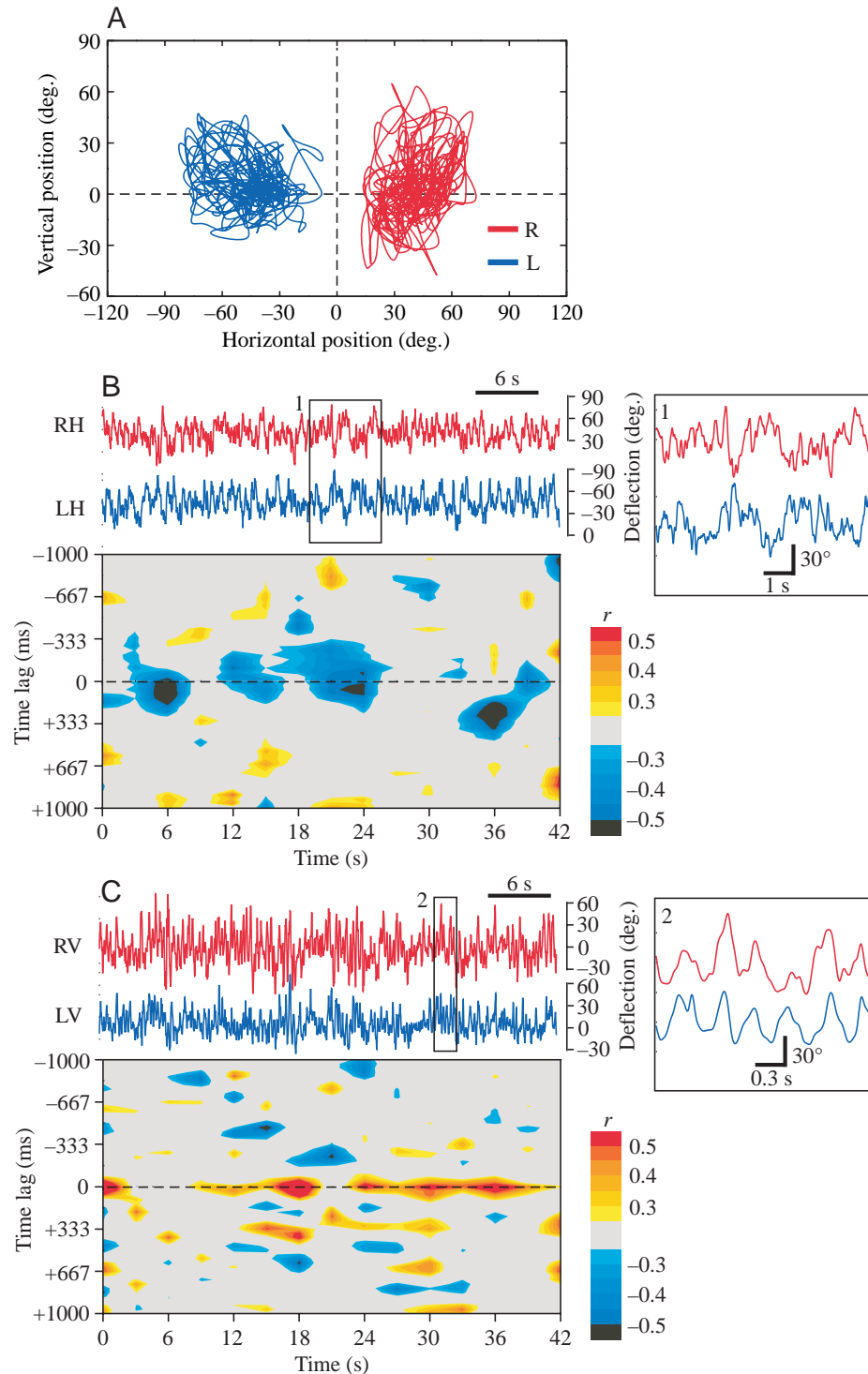


Fig. 10. Cross-correlations between right and left antennal movements in walking. The data were from the same animal as in Fig. 9. (A) Trajectories of both antennae were still random, and their scanning areas were smaller than those seen during pausing (cf. Fig. 9A). (B) Cross-correlation for horizontal components. Significant negative correlations existed close to the zero time lag as relatively compact spreads along the ordinate (compare with Fig. 9B). Inset shows expansion of the boxed area (1). Though individual small waves did not exactly synchronize with each other, coordination is recognizable in both larger and slower components. (C) Cross-correlation for vertical components. Significant positive correlation spots were continuously observed over the map at zero time lag as sharp peaks. Expansion of the boxed area (2) shows synchronization of the pair of waves at 2–3 Hz. Note the time scale difference from Inset 1.

1.5–3.5 Hz in pausing. When walking, the major peaks were distributed over wider range up to 4.5 Hz. These characteristics of the vertical component were similar to those in the normal antenna (compare with Fig. 5). The same analyses on the horizontal component revealed only minute peaks, but their distribution pattern closely resembled that for the vertical component (data not shown). This effect is simply due to contamination by the vertical component, probably because the rotation axis of the S–P joint is not precisely horizontal in the present coordinate system. In the H–S joint-free condition (Fig. 8B), spectra for both the horizontal and vertical deflections had their main components at lower frequency (<1 or <2 Hz). Spectral patterns for the horizontal deflection were analogous to those in the normal condition for both locomotory modes (see Fig. 5).

Couplings between the right and left antennal motor systems

It appeared from inspection of the time courses of antennal movements that the right and left horizontal components are coordinated in anti-phase (Fig. 3), but the exact details are still unclear. For vertical deflections, no obvious rule could be deduced from the time courses of a pair of antennae. To investigate whether movement of the pair of antennae is coordinated or not, cross-correlation analyses were performed. Correlation coefficients, whose values should indicate the extent of coupling, were calculated every 3 s in order to investigate the dynamic feature of the coupling.

Fig. 9 shows an example from the pausing state. The trajectory patterns for both antennae were rather random, as in most cases (Fig. 9A). In the correlation map, significant negative correlations in the horizontal component appeared as spots from place to place (Fig. 9B). The correlation spots were relatively broad along the time lag axis, and deviated from the center (zero time lag). The timing of the negative correlations seemed to correspond to large amplitude movements, where both antennae move

in the same direction. These suggest that the horizontal motor systems couple between the right and left sides for large amplitude movement in an anti-phase manner. By contrast, the vertical components exhibited significant correlations only very sparsely on the correlation map, indicating that there is little or no correlation between the right and left vertical motor systems during pausing (Fig. 9C).

When the searching mode changed to walking, the areas scanned by both antennae decreased and shifted to lower position, but the trajectory pattern was still disordered (Fig. 10A). A cross-correlation analysis for the horizontal components revealed that significant negative correlations were concentrated at zero time lag (Fig. 10B), compared with the correlation map in pausing (Fig. 9B). This suggests that the horizontal deflections in both antennae coordinate more precisely with each other in anti-phase. A more notable change was observed in the correlation map for vertical components: significant positive correlations occurred continuously at zero time lag, with sharp peaks (Fig. 10C). The result strongly suggests that the tight couplings between the right and left vertical systems were in orthophase. Actually, the waves for the vertical component were strictly synchronized between both antennae (see inset at Fig. 10C). In the correlation map, there were some places that positive (yellow to red) and negative (blue to black) spots appeared to alternate along the ordinate (time lag axis), which may represent synchronized oscillations of both antennae at a constant frequency (about 3 Hz in the case of Fig. 10C).

Correlation indices (CIs) were calculated for the horizontal and vertical components in both pausing and walking, and their differences were tested statistically between both states (Table 1). Since coupling was correlated most clearly at zero time lag, the analysis was restricted to the correlation coefficient there. The results revealed for both components that there were significant differences between pausing and

walking (Mann–Whitney *U*-test, $P < 0.05$). Thus the couplings between the right and left antennal motor systems may alter, depending on the mode of searching behavior.

An application of coupling analyses to the joint-manipulated specimens appears to provide more detailed information about the searching mode-dependency of antennal movement, in that the dependency could be specified as kinematics in each joint. We performed cross-correlation analyses for four samples in each searching mode and for each joint treatment, but for both the horizontal and vertical components these failed to detect any CI differences between the two searching modes (Mann–Whitney *U*-test, $P > 0.05$). Although most of the treated animals exhibited unusual behavior, as described above, we carefully selected samples that could be regarded as normal. In our previous report (Okada and Toh, 2000), similar behavioral changes were described in animals with immobilized antennal joints. Such treatment may affect the antennal motor system, and thus its coordinated outputs would be impaired even if an animal appeared to be behaving naturally.

Discussion

Spatio-temporal characterization of the antennal and head movements

The tip of antenna is operated by the two joints at its base and by the neck. As integrated antennal movements involve at least six degrees of freedom in total, they are considered to be highly complicated. However, since deflections in the head roll were relatively small during searching (at most 15°), movement of the antennal tip could be reduced simply to the horizontal and vertical components in the antennal joints and the head.

The joint-manipulation experiments provided information on the temporal characteristics of the horizontal and vertical components in normal antennae. When only the S–P joint could move in the nearly vertical plane, a major component of the power-spectrum appeared at faster frequency (see examples in Fig. 7B; < 3.5 Hz in pausing, < 4.5 Hz in walking). On the other hand, when only the H–S joint was free, spectral peaks for the horizontal component were observed mainly at lower frequency in both states (Fig. 8B; < 2 Hz in pausing, < 1 Hz in walking). These spectral patterns in joint-manipulated conditions closely resembled those seen in the normal condition (compare with the corresponding spectra in Fig. 5). It is therefore likely that the horizontal movement of antennae originates almost entirely from the H–S joint, the fast vertical movement (presumably at > 2 Hz) mainly from the S–P joint, and the slow vertical one (at < 2 Hz) from both the S–P and H–S joints.

Investigations of spatial distribution about the antennal joints and the head clarified the extent to which these two components contribute to the total displacement of antennae. Large amplitude movements in the head yaw and pitch components were more frequent in the pausing state, which may serve for intermittent careful probing of the surroundings. Roll movements were particularly observed during leg grooming: large roll deflections

Table 1. *Correlation indices between right and left antennae for horizontal and vertical deflections*

Animal (<i>N</i> =10)	Horizontal		Vertical	
	Pausing	Walking	Pausing	Walking
S4E	–0.201	–0.274	–0.098	0.147
S4F	–0.152	–0.138	0.103	0.370
S8A	–0.195	–0.258	–0.032	0.448
S8B	–0.139	–0.282	0.011	0.089
S8C	–0.226	–0.266	0.262	0.342
S8D	–0.267	–0.398	0.212	0.136
S8F	–0.195	–0.320	0.083	0.265
S8I	–0.132	–0.336	0.193	0.243
S8L	–0.434	–0.577	0.254	0.117
S8S	–0.176	–0.188	0.054	0.276
Mean	–0.212	–0.304	0.104	0.243
<i>P</i> *	$P < 0.05$		$P < 0.05$	

The CI (correlation index) was defined as the average of the correlation coefficients at zero time lag for a series of 3 s periods.

*Mann–Whitney *U*-test.

often amounted to 90° from the dorso-ventral plane (J.O. and Y.T., personal observation). From our experience to date, the temporal relationship between the three head components remains unclear, and warrants further analysis.

Spatial characteristics of the antennal movements were represented in their trajectories. The most striking feature was that the area scanned by an antenna altered depending on the mode of searching. In the intermittent pausing state, the antenna covered a relatively large area. This global scanning might contribute to the sensitive discovery of chemical and physical cues from resources at various places. Head movement also participated in the careful scanning as mentioned above. While in the walking state, the scanning area narrowed, mainly because of a decrease in the horizontal deflection, and the entire vertical position lowered. The joint-immobilization experiments specified in part which joint is related to these searching-mode dependencies of antennal movement. When only the H-S joint was free, the horizontal angular range during walking significantly narrowed, as with normal antennae (Fig. 8C), suggesting that the H-S joint is concerned in the mode-dependency. On the other hand, in the S-P joint-free condition, the central position of the vertical component lowered significantly by about 25° during walking (Fig. 7C). Similarly, the lowering of vertical position (about 15° in amplitude) was observed in the H-S joint-free condition (Fig. 8C). These results imply that both the S-P and H-S joints participated in the searching-mode dependency of the vertical central position.

An observation that the cockroach antennae tend to point forward at a low angle was described in an earlier unpublished thesis study (McCoy, 1985, 1986). Insects when walking generally point their antennae to the front in order to detect forthcoming gaps or obstacles (Pelletier and McLeod, 1994; Horseman et al., 1997; Camhi and Johnson, 1999; Dürr et al., 2001). In the cockroach, antennal oscillation seemed to be suppressed according to the animal's locomotion. Actually, cockroaches almost fixed their antennae anteriorly during running or flight (J.O. and Y.T., personal observation). However, Yagodin and Kovbasa (1984) reported in *P. americana* that a vibration-like activity of the antenna with small amplitude (around 1 mm) and fast frequency (3–10 Hz) is still observable even in the flight. They also described from joint-fixation experiments that self-stimulation of unidentified pedicellar mechanoreceptors by the active vibration of antennae may be essential for maintenance of prolonged flight. It is therefore possible that feedback from mechanoreceptors located at the pedicel or its adjacent segments, such as the campaniform sensilla, chordotonal organ and Johnston's organ, affects the locomotion center and consequently or simultaneously the antennal motor center as well. Horseman et al. (1997) showed in the cricket that the transection of the ipsilateral circumesophageal connective abolishes antennal oscillation in walking and the prolonged forward positioning of antennae during flight. The authors concluded that the brain may be insufficient to generate antennal movements, and that ascending inputs from the subesophageal and/or thoracic

ganglia are important for the behavior-specific antennal movement and posture. Though we have not applied such treatment to cockroaches, it might give some clues for locomotion-dependent control of antennal movements and gross localization of its center in the CNS.

Regularity in antennal trajectory patterns

In most cases analyzed here, antennal trajectories showed no patterned regularity in either pausing or walking; however, in a few cases, the loop-like pattern occurred (Fig. 6). It is still unclear whether the loop-like pattern was induced by some unpredictable external stimuli or by unknown endogenous factors. We found recently in independent experiments that the loop-like pattern is related to tactile orientation behavior (J. Okada and Y. Toh, unpublished). When a blinded animal encounters a stable object with its antennae while searching, it may touch the object repeatedly with its antennae and try to approach it (Okada and Toh, 2000). This positive thigmotaxis may be useful for finding appropriate habitats such as narrow crevices. Our recent finding in the tethered-walking condition was that once animals have released tactile orientation behavior, many of them exhibit the loop-like pattern in their antennal trajectory, which continues for more than a few minutes even in the absence of tactile objects. It is thus likely that the antennal trajectory pattern may reflect motivation of cockroaches.

The loop-like pattern could be subdivided into three basic pattern units, the loop, arch and vertical line (see insets in Fig. 6B2 for examples). The loop and arch patterns are presumably attributable to coordination between the horizontal and vertical deflections at the antennal joints. We surmise that the pattern generation arises from coordinated activities between the vertical motor system in the S-P joint and the horizontal one in the H-S joint. To determine the extent of the coupling between these motor systems, cross-correlation analysis is useful, but we hesitated to use this analysis because the rotation axes of the joints were not precisely horizontal or vertical. If cross-correlation analyses were applied to the ipsilateral horizontal and vertical components using the present measuring system, the results would contain many artefacts, indicating excessively large correlations. An alternative method for avoiding such errors would be to record activities of antennal muscles or motor nerves and to perform cross-correlations. This should be a more reliable procedure for understanding the central mechanism for the pattern generation directly, and is currently under investigation using muscarinic agonists as the antennal rhythm generator (for preliminary results, see Okada and Toh, 2003).

Couplings between the right and left antennal motor systems

Cross-correlation analyses on searching cockroaches showed coupling strengths between the right and left antennal motor systems in different behavioral modes. The horizontal motor system comprises a pair of abductor and adductor muscles for the scape. The vertical motor system involves two elements: a levator muscle for the scape and a pair of levator

and depressor muscles for the pedicel. The center controlling these antennal motor systems is located at the dorsal deutocerebrum (dorsal lobe) in many insects (Rospars, 1988; Homberg et al., 1989). This area also receives primary afferents of the antennal mechanoreceptors, so is called alternatively the antennal mechanosensory and motor center (AMMC). Moreover, in cockroaches, descending interneurons related to the antennal mechanoreception send their dendrites to the dorsal lobe (Burdohan and Comer, 1996) and crickets (Gebhardt and Honegger, 2001). It may thus be a general feature in insects that the dorsal lobe is regarded as the center for both integrating antennal mechanosensory information and generating outputs for appropriate antennal motor patterns.

Anti-phase couplings between the right and left horizontal systems were consistently observed regardless of the searching mode (pausing/walking). The coupling strength was significantly larger during walking than in pausing. Couplings between the antennal horizontal component and the head yaw could not be examined quantitatively in the present study because large yaw deflections were rare in the data sampled. However, whenever sufficiently large yaw deflections occurred, both antennae pointed in the same direction as the head. In addition, large deflections in the antennae and head often accompanied intended turns of the body. These suggest that the antennal horizontal motor systems in both sides, which are probably composed of antagonistic pairs of the abductor and adductor muscles for the scape, couple to the head yaw system as well as to the steering system for walking. The anti-phase relationship in the horizontal components of both antennae has been described in cockroaches (McCoy, 1985, 1986; Okada et al., 2002), crickets (Honegger, 1981; Horseman et al., 1997) and stick insects (Dürr et al., 2001) as well as in crustaceans (Zeil et al., 1985). Thus, this might be common to arthropods for active sensing of the physical environment by antennation.

Coupling between the right and left vertical motor systems changed dynamically depending on the animal's behavioral state. When an animal paused, the coupling was relatively loose, but became coherent during walking. The mode of coupling was orthophase: dorsoventral movements were synchronized between the right and left antennae. The frequency of synchronization was relatively fast at more than 2 Hz. Spectral analyses about the joint-manipulated animals revealed that vertical deflections of the H-S joint contained few fast components at >2 Hz in its FFT spectra (Fig. 8B). Thus, the synchronized movement may originate mainly from the S-P joints. As far as we observed in the video images, it is unlikely that the antennal vertical rhythmicity is always caused by contact with the ground, which is consistent with observations in the stick insect (Dürr et al., 2001). Although some proprioceptors in the antenna may actually be involved more-or-less in its oscillatory movements (e.g. Okada et al., 2002), the synchronization of the vertical systems may derive essentially from the antennal motor center in the dorsal deutocerebrum, independent of the proprioceptive influence.

Temporal relationships were examined between antennae

and legs. Coupling analyses detected significant correlations only in one walk out of 14 (tested in 14 animals). We therefore conclude that there is little evidence for coupling between the antennae and the legs, as suggested earlier by McCoy (1985, 1986). Coordination between the antenna and leg has been described in crickets (Horseman et al., 1997), stick insects (Dürr et al., 2001) and crayfish (Sandeman and Wilkens, 1983), but is absent in locusts (Saager and Gewecke, 1989). This inconsistency, including the present results, may simply reflect species differences. Another interpretation is that the inconsistency is attributable to methodological differences: some studies used quantitative analyses, but others were based just on visual observation. It is possible that application of reliable statistical analyses might yield different results.

How is the coordination or synchronization of antennal movements generated? Our survey is largely behavioral, so there is no physiological evidence bearing upon the question. A simple speculation for the phenomenon is that the antennal motor systems receive bilateral oscillatory inputs in common from an unknown locomotion-related center, and coordination or synchronization may be generated as the result. Another hypothesis is that the antennal motor systems in both sides themselves interact directly with each other, and entrainment arises between both antennae. This problem remains to be examined physiologically, and should be addressed initially with neural mechanisms in the dorsal deutocerebrum.

What is the purpose of coordination or synchronization in both antennae? The phenomenon was pronounced in the walking state. Because both antennae are aligned more in parallel during walking, antennation in this situation probably increases the opportunity to detect objects in front with both antennae simultaneously. This is more effective for gaining information about objects than using only a single antenna; simultaneous activation of both antennae could be concerned in identification of an object, and consequently in perception of its size.

We proposed in a previous study that cockroaches recognize the orientation of stable objects by antennation (Okada and Toh, 2000). A cluster of mechanosensitive hairs at the scape (the scapal hair plate) is one potential candidate to perceive the orientation of the antenna itself, and in turn the direction of objects. In addition, it was recently reported that cockroaches can presumably discriminate texture or shape of tactile objects by antennation (Comer et al., 2003), and these behavioral studies suggest that mechanoreception by the active antenna involves a variety of tactile modalities. We surmise that searching by antennal scanning is an alternative strategy to vision for constructing a map of the physical world in the brain, which is especially important for nocturnal insects.

The authors are grateful to Dr S. R. Shaw (Dalhousie University, Halifax, Canada) for reading the manuscript and giving valuable comments, and to Mr J. Wakamatsu and Ms H. Nagata for their helpful assistance. This study was supported in part by a Grant-in-Aid for Scientific Research on Priority Areas (A) from the Ministry of Education, Culture,

Sports, Science and Technology of Japan (12048216), a Grant-in-Aid for Young Scientists from the Japan Society for the Promotion of Science (14740463), and the Narishige Zoological Science Award.

References

- Altner, H. and Prillinger, L. (1980). Ultrastructure of invertebrate chemo-, thermo, and hygroreceptors and its functional significance. *Int. Rev. Cytol.* **67**, 69-139.
- Bell, W. J. (1991). *Searching Behaviour: The Behavioural Ecology of Finding Resources*. London: Chapman and Hall.
- Burdohan, J. A. and Comer, C. M. (1996). Cellular organization of an antennal mechanosensory pathway in the cockroach, *Periplaneta americana*. *J. Neurosci.* **16**, 5830-5843.
- Camhi, J. M. and Johnson, E. N. (1999). High-frequency steering maneuvers mediated by tactile cues: antennal wall-following in the cockroach. *J. Exp. Biol.* **202**, 631-643.
- Chapman, R. F. (1982). Chemoreception: The significance of receptor numbers. *Adv. Insect Physiol.* **16**, 247-356.
- Comer, C. M., Parks, L., Halvorsen, M. B. and Breese-Terteling, A. (2003). The antennal system and cockroach evasive behavior. II. Stimulus identification and localization are separable antennal functions. *J. Comp. Physiol. A* **189**, 97-103.
- Dürr, V., König, Y. and Kittmann, R. (2001). The antennal motor system of the stick insect *Carausius morosus*: anatomy and antennal movement pattern during walking. *J. Comp. Physiol. A* **187**, 131-144.
- Ehmer, B. and Gronenberg, W. (1997a). Antennal muscles and fast antennal movements in ants. *J. Comp. Physiol. B* **167**, 287-296.
- Ehmer, B. and Gronenberg, W. (1997b). Proprioceptors and fast antennal reflexes in the ant *Odontomachus* (Formicidae, Ponerinae). *Cell Tissue Res.* **290**, 153-165.
- Erber, J., Pribbenow, B., Bauer, A. and Kloppenburg, P. (1993). Antennal reflexes in the honeybee: Tools for studying the nervous system. *Apidologie* **24**, 283-296.
- Erber, J., Pribbenow, B., Grandy, K. and Kierzek, S. (1997). Tactile motor learning in the antennal system of the honeybee (*Apis mellifera* L.). *J. Comp. Physiol. A* **181**, 355-365.
- Gebhardt, M. and Honegger, H.-W. (2001). Physiological characterisation of antennal mechanosensory descending interneurons in an insect (*Gryllus bimaculatus*, *Gryllus campestris*) brain. *J. Exp. Biol.* **204**, 2265-2275.
- Homberg, U., Christensen, T. A. and Hildebrand, J. G. (1989). Structure and function of the deutocerebrum in insects. *Ann. Rev. Entomol.* **34**, 477-501.
- Honegger, H.-W. (1981). A preliminary note on a new optomotor response in crickets: Antennal tracking of moving targets. *J. Comp. Physiol.* **142**, 419-421.
- Horseman, B. G., Gebhardt, M. J. and Honegger, H.-W. (1997). Involvement of the suboesophageal and thoracic ganglia in the control of antennal movements in crickets. *J. Comp. Physiol. A* **181**, 195-204.
- Keil, T. A. and Steinbrecht, R. A. (1984). Mechanosensitive and olfactory sensilla of insects. In *Insect Ultrastructure*, vol. 2 (ed. R. C. King and H. Akai), pp. 477-516. New York: Plenum Press.
- Lee, J.-K. and Strausfeld, N. J. (1990). Structure, distribution and number of surface sensilla and their receptor cells on the olfactory appendages of the male moth *Manduca sexta*. *J. Neurocytol.* **19**, 519-538.
- Lent, D. D. and Kwon, H.-W. (2004). Antennal movements reveal associative learning in the American cockroach *Periplaneta americana*. *J. Exp. Biol.* **207**, 369-375.
- McCoy, M. M. (1985). Antennal movements of the American cockroach *Periplaneta americana*. PhD thesis, University of Kansas.
- McCoy, M. M. (1986). Antennal movements of the American cockroach *Periplaneta americana*. *Diss. Abst. Int.* **46**, 3327-B.
- Norris, D. and Chu, H.-M. (1974). Morphology and ultrastructure of the antenna of male *Periplaneta americana* as related to chemoreception. *Cell Tissue Res.* **150**, 1-9.
- Okada, J., Hinoue, K. and Toh, Y. (2001). 3-D analysis of spontaneous antennal movements in the cockroach *Periplaneta americana*. *Zool. Sci.* **18** Suppl, 92 (Abstr).
- Okada, J., Kanamaru, Y. and Toh, Y. (2002). Mechanosensory control of antennal movement by scapal hair plates in the American cockroach. *Zool. Sci.* **19**, 1201-1210.
- Okada, J. and Toh, Y. (2000). The role of antennal hair plates in object-guided tactile orientation of the cockroach (*Periplaneta americana*). *J. Comp. Physiol. A* **186**, 849-857.
- Okada, J. and Toh, Y. (2002). A three dimensional analysis of antennal movement in the tactile orientation behavior of American cockroaches. *Zool. Sci.* **19**, 1463 (Abstr).
- Okada, J. and Toh, Y. (2003). Antennal movements in cockroaches: A comparison between the spontaneous and drug-induced patterns. *Zool. Sci.* **20**, 1572 (Abstr).
- Pelletier, Y. and McLeod, C. D. (1994). Obstacle perception by insect antennae during terrestrial locomotion. *Physiol. Entomol.* **19**, 360-362.
- Rospars, J. P. (1988). Structure and development of the insect antenno-deutocerebral system. *Int. J. Insect. Morphol. Embryol.* **17**, 243-294.
- Rust, M. K., Burk, T. and Bell, W. J. (1976). Pheromone-stimulated locomotory and orientation responses in the American cockroach. *Anim. Behav.* **24**, 52-67.
- Saager, F. and Gewecke, M. (1989). Antennal reflexes in the desert locust *Schistocerca gregaria*. *J. Exp. Biol.* **147**, 519-532.
- Sandeman, D. C. and Wilkens, L. A. (1983). Motor control of movements of the antennal flagellum in the Australian crayfish, *Euastacus armatus*. *J. Exp. Biol.* **105**, 253-273.
- Schafer, R. and Sanchez, T. V. (1973). Antennal sensory system of the cockroach, *Periplaneta americana*: Postembryonic development and morphology of the sense organs. *J. Comp. Neurol.* **149**, 335-354.
- Schaller, D. (1978). Antennal sensory system of *Periplaneta americana* L.: Distribution and frequency of morphologic types of sensilla and their sex-specific changes during postembryonic development. *Cell Tissue Res.* **191**, 121-139.
- Schneider, D. (1964). Insect antennae. *Ann. Rev. Entomol.* **8**, 103-122.
- Seelinger, G. and Tobin, T. R. (1981). Sense organs. In *The American Cockroach* (ed. W. J. Bell and K. G. Adiyodi), pp. 217-245. London: Chapman and Hall.
- Steinbrecht, R. A. (1984). Chemo-, hygro, and thermoreceptors. In *Biology of the Integument*, vol. 1 (ed. J. Bereiter-Hahn, A. G. Matoltsy and K. S. Richards), pp. 523-553. Berlin: Springer-Verlag.
- Suzuki, H. (1975). Antennal movements induced by odour and central projection of the antennal neurones in the honey-bee. *J. Insect Physiol.* **21**, 831-847.
- Toh, Y. (1977). Fine structure of antennal sense organs of the male cockroach, *Periplaneta americana*. *J. Ultrastruct. Res.* **60**, 373-394.
- Yagodin, S. V. and Kovbasa, S. I. (1984). The flight maintenance mechanisms in the cockroach *Periplaneta americana* L. *J. Comp. Physiol. A* **155**, 697-712.
- Ye, S., Leung, V., Khan, A., Baba, Y. and Comer, C. M. (2003). The antennal system and cockroach evasive behavior. I. Roles for visual and mechanosensory cues in the response. *J. Comp. Physiol. A* **189**, 89-96.
- Zacharuk, R. Y. (1985). Antennae and sensilla. In *Comprehensive Insect Physiology, Biochemistry and Pharmacology*, vol. 6 (ed. G. A. Kerkut and L. I. Gilbert), pp. 1-69. Oxford: Pergamon Press.
- Zeil, J., Sandeman, R. and Sandeman, D. C. (1985). Tactile localization: The function of active antennal movements in the crayfish *Cherax destructor*. *J. Comp. Physiol. A* **157**, 607-617.