

THE PARS INTERCEREBRALIS NEURONE PI(2)5 OF LOCUSTS: CONVERGENT PROCESSING OF INPUTS REPORTING HEAD MOVEMENTS AND DEVIATIONS FROM STRAIGHT FLIGHT

By KLAUS HENSLER

Zoologisches Institut, Rheinsprung 9, 4051 Basel, Switzerland

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Summary

The pars intercerebralis neurone PI(2)5 of *Locusta migratoria* is a descending large-field deviation detector (DN), projecting from the brain to the fused abdominal ganglia. It responds with bursts of action potentials (1) to specific movements of an artificial horizon, simulating deviations from course (preferred deviations are roll and yaw to the side of the axon, and pitch-up), and (2) to proprioceptive input from neck receptors signalling head movements in either direction. During compensatory head movements both inputs act together so that the deficit in visual information is balanced by proprioceptive input.

The number of spikes elicited by roll deviations depends on the sector of the visual yield initially covered by the horizon (sector specificity), increasing the more the horizon is oriented in the visually preferred direction. Hence, sector specificity involves information about the absolute orientation with respect to the horizon. The number of spikes depends further on the roll position of the head with respect to the prothorax. The response is amplified when the head is oriented in the visually preferred direction, and is attenuated when the head is oriented in the visually antipreferred direction. Visual and proprioceptive modulatory effects balance each other so that sector specificity relates to the body (i.e. the aerodynamic organs) even when head and body are rotated against each other.

During flight, the excitability of PI(2)5 is further influenced by tonic inhibition from frontal wind (no directionality), which is overridden by tonic excitation of central origin.

Introduction

To maintain course and orientation in space, flying locusts must correct for involuntary deviations caused by turbulence or by inaccuracies of their own motor output. Deviations are associated with changes of the wind direction which can be perceived by mechanoreceptors on different parts of the body (sense organs of wings: Wendler, 1983; Elson, 1987; sternal hairs: Pflüger, 1984; cerci: Heinzel, 1983; Boyan *et al.* 1986). However, the most important deviation detectors are concentrated on the head; namely, the compound eyes, the ocelli, the wind-

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sensitive hairs and the antennae (Goodman, 1965; Taylor, 1981*a,b*; Camhi, 1969; Gewecke, 1974; Arbas, 1986). These receptors provide input to a population of plurisegmental deviation detector neurones (DNs) descending from the brain to at least the fused abdominal ganglia (some authors use the abbreviation DN for descending neurones in general; here it is used only in the defined sense). Projections of the DNs extend into the dorsal neuropile of each ganglion (see Griss & Rowell, 1986). There the information is processed and relayed to motoneurones (MNs) to elicit correctional steering movements of wings, abdomen and legs, and compensatory movements of the head (reviewed by Rowell, 1988).

Four of these DNs have been described in detail. The tritocerebral commissure giant neurone (TCG; Bacon & Tyrer, 1978) responds to changes of wind direction, favouring ipsilateral yaw (Möhl & Bacon, 1983), and the multimodal neurones DNI, DNC and DNM respond to roll, yaw and pitch deviations of the panorama, each preferring a different direction. They are driven by convergent, interactive inputs from the eyes, the ocelli and the wind-sensitive head hairs (Rowell & Reichert, 1986). Their activity is gated, adapted to the phase of flight, and distributed to wing MNs, by a population of thoracic interneurones (Reichert & Rowell, 1985), thus forming a sort of 'autopilot' that automatically corrects for deviations from straight flight.

This model leaves out the observation that, like many visually oriented animals, locusts minimize retinal slip due to deviations from course (thus also changes in wind-flow pattern) by compensatory head movements (Taylor, 1981*a,b*; Camhi, 1970). In other words, the orientation of the sense organs detecting deviations is not fixed with respect to the muscles performing steering. Information about course deviations is thus partitioned between two sensory sources: exteroceptive input reporting the orientation of the head with respect to the outer world, and proprioceptive input signalling the relative orientation of head and body. Steering must take account of both inputs.

Convergence of these inputs at the level of single neurones has only been described for the thoracic neurone 5AA (Elson, 1987). 5AA codes for ocellar input, wing strain and head movements, but it is still not clear how these inputs combine during normal behaviour. The present paper characterizes the pars intercerebralis neurone PI(2)5 of locusts under stimulus conditions close to those expected during normal flight. PI(2)5 is the first identified locust DN shown to receive convergent visual and neck receptor inputs. Its responses to light, movements of an artificial horizon, head movements and frontal wind are described for locusts at rest and during flight activity. The complex, interactive processing of these inputs is explained, and a model is presented of how neck receptor input is involved in corrective flight steering.

Materials and methods

Preparation and experimental setup

Experiments were performed at 24–30°C on more than 35 adult *Locusta*

migratoria of either sex from a laboratory culture or from a commercial supplier. The animals were dissected using a modified version of the method described by Robertson & Pearson (1982): legs and wings were removed, the pterothorax and two segments of the abdomen were cut open along the dorsal midline, and the animals were pinned onto a piece of balsa wood, the head protruding over the front edge (Fig. 1). The thoracic ganglia were exposed by removing the gut and some leg muscles. Care was taken not to damage the anterior prothorax and the neck joint in order to guarantee the function of neck muscles and neck proprioceptors. Movements at the joint between pro- and pterothorax were prevented by stabilizing it with wax. Head movements around the longitudinal body axis (head rolling) were measured with a capacitance device (Sandeman, 1968; see legend to Fig. 1). Passive head rolling was produced with a servomotor using the antenna of the Sandeman device as a lever. The motor was mounted on a manipulator. The end of its axis was supplied with a fork fitting the antenna which could be coupled or uncoupled during the experiment, allowing or preventing active head rolling. In the coupled state, voluntary yaw and pitch movements of the head were still possible.

The animals were positioned in front of an artificial horizon, a hemisphere with

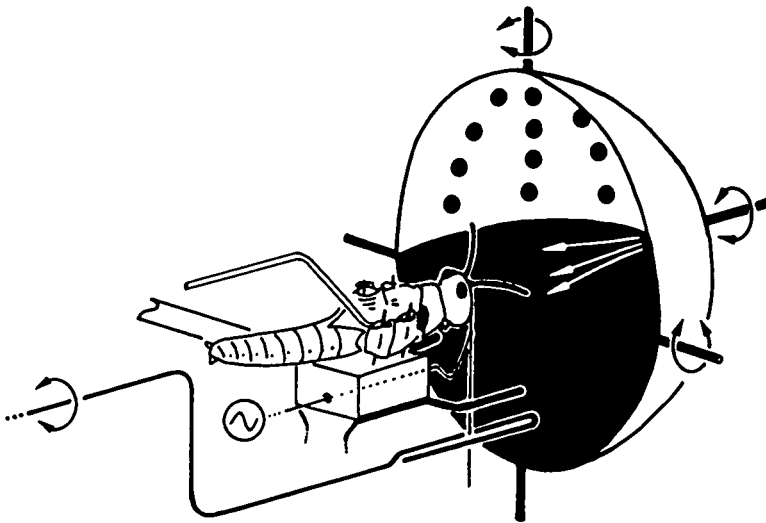


Fig. 1. Schematic outline of the experimental setup. The dissected locust was pinned onto a piece of balsa wood and mounted with the head at the centre of rotation of an artificial semihorizon. The horizon could be rotated by means of servomotors around the roll, yaw and pitch axis by 56° , 30° and 14° , respectively, in each direction. Wind could be blown onto the locust's head through a 12 mm hole in the middle of the horizon (white arrows). The head was moved passively around the roll axis using an insect pin glued vertically onto the head as a lever. The pin also served as the antenna of a position transducer, being connected to a sine-wave generator. An electronic device transformed the signals induced in two sensors on the lower edges of the balsa block into a voltage level which was proportional to the roll position of the head. For further information see text.

a diameter of 70 mm (Rowell & Reichert, 1986). The lower half was painted black and the translucent upper half was structured with black dots. It was illuminated by a small tungsten bulb from the rear producing a maximum luminance of 300 cd m^{-2} (measured between 450–650 nm). Visual input from behind the animal was prevented by covering the rear halves of the compound eyes with black enamel and by keeping the windowless laboratory as dark as possible. Experiments requiring darkness were made immediately after switching off a bright light. This minimized adaptation by the locust to background light emanating from sources in the electronic equipment.

The horizon could be rotated around the roll, yaw and pitch axes by means of servomotors, controlled by hand *via* potentiometers or by a waveform generator. Non-laminar wind (up to 4 m s^{-1}) provided by a hair-dryer could be blown onto the locust's head through a 12 mm diameter tube in the middle of the horizon. In a few experiments wind was blown onto the cerci from different directions through a 10 mm tube at a distance of 4 cm.

Recording and staining techniques

Intracellular recordings were made with glass microelectrodes filled at the tip with 5 % Lucifer Yellow (Stewart, 1978), and backfilled with 0.5 mol l^{-1} lithium acetate. PI(2)5 was mostly penetrated in the meso- or methathoracic ganglia or in a thoracic connective. Both ganglia were supported using a steel platform. In a few cases, recordings were made from the prothoracic ganglion, which was stabilized against head movements by fixing it with tissue glue onto a spoon equipped with slots for the connectives and the lateral nerves (Hensler, 1986). Movement artefacts due to the action of the direct flight muscles were prevented by transecting the motor nerves (nerves 3, 4, 5). Activity in one of the indirect wing depressors (M81 or M112) served as an indicator for fictive flight.

The activity of PI(2)5 was recorded using conventional methods. Data were stored on tape for subsequent evaluation. Lucifer Yellow was injected for up to 2 h with hyperpolarizing current of 8–10 nA. In no case was this enough to stain the entire cell. It was especially difficult to fill the brain structures. This problem was overcome by *in vivo* visualization of the stained parts by illumination with blue light from a He–Cd laser (see Reichert & Krenz, 1986). It was thus possible to repenetrate the cell repeatedly at increasingly anterior locations. The light intensity was low and did not damage the cell, as indicated by the unaltered amplitude of action potentials. To check whether the same neurone was repenetrated (and not another one lying exactly above or under it), the resulting histological preparations were viewed from different perspectives. Although time-consuming (5–6 h), this method could successfully be applied to neurones with superficial, large axons.

Successful fills were fixed in 4 % formaldehyde in phosphate buffer (pH 7), dehydrated in ethyl alcohol and cleared in methylsalicylate for subsequent viewing under a fluorescence microscope. The structure with respect to internal landmarks of the ganglion (Tyrer & Gregory, 1982) was revealed by embedding suitable

preparations in soft epoxy resin or in paraffin and cutting transverse 30 μm sections.

Evaluation of data

Many conclusions in this paper are based on the quantitative evaluation of responses to different combinations of stimuli. Under each condition, stimuli were repeated 10–20 times, usually at intervals of 3–7 s. One programme contained 4–20 such sequences. Sometimes the sequence lasted for more than 30 min and contained up to 400 stimulus presentations. Therefore, measures had to be taken to minimize effects of changed responsiveness due to habituation/dishabituation or to other factors (see last section of the Results). A sequence was rejected if it showed sudden, substantial changes of the response despite unchanged stimulus conditions. Effects of initial, strong habituation were avoided by repeating stimuli several times before starting measurements, i.e. measurements were all derived from habituated preparations. Further, the mean values of each sequence were normalized with respect to control experiments repeated every 2–4 sequences. To this end, they were plotted in temporal sequence and the 100 % value appropriate to each was determined by interpolating linearly between the neighbouring controls (see dotted line, Fig. 8). In some cases the effects of habituation were negligible or small. Then only the initial and terminal control values are shown.

Definitions

Following the practice of Griss & Rowell (1986) the terms ipsi- and contralateral are used with respect to the recorded axon (unless otherwise stated). Movements of the artificial horizon relative to the fixed locust (thus simulating visually a deviation from course) are designated as experienced by the animal. For example, a horizon movement to the right is described as deviation (of the locust) to the left (see Rowell & Reichert, 1986). In a strict sense deviations are movements away from the normal flight position. For practical reasons this term is used here for horizon movements starting at any position in the visual field.

Results

Anatomy

Many descending neurones (DNs), including PI(2)5, have quite similar thoracic structures and are morphologically identifiable only by their anatomy in the brain (Griss & Rowell, 1986; K. Hensler, unpublished observations). Because of the laborious procedure associated with staining PI(2)5 in the brain (see Materials and methods) this was done only four times. The neurones were identified physiologically (see Discussion) before they were stained. In all four cases the branching patterns were uniform and congruent with those of a neurone called PI(2)5 by Williams (1975), who described its structure in the brain of *Schistocerca gregaria*. He defined it as the fifth of a group of six neurones, the cell bodies of which are tightly packed in the middle part of the pars intracerebralis.

The entire structure of PI(2)5 is shown in Fig. 2. In the protocerebrum, contralateral to the axon, numerous branches extended laterally just below the dorsal surface (section 2). A posterior branch projected into the dorsal and median portions of the deutocerebrum (section 3 right). Having crossed the midline the axon sent short branchlets into the dorsal deutocerebrum (section 3 left). In contrast to the other smooth branches of the brain these were of beaded appearance.

Posterior to the brain the axon descended in the median dorsal tract (MDT), at least as far as the fused abdominal ganglia 1–3. There its diameter decreased from 10–15 μm to less than 2 μm . In a few fills it extended further into the abdominal connective (A3–A4) for several hundred micrometres. It is not known whether it ended there or was simply too thin to be filled more completely. The morphology in the ganglia posterior to the brain was uniform. Lateral branches projected predominantly dorsally, medial branches projected more ventrally. These branches were beaded. In the suboesophageal ganglion projections were confined to the labial and maxillar neuromeres.

The smooth appearance of the branches on the soma side suggests input regions, the beaded appearance of all other branches suggests output regions (see Peters *et al.* 1986, although beads do not indicate output synapses in locust motoneurons, Watson & Burrows, 1982). This view is supported by physiological measurements. Subthreshold activity was not seen while recording at different locations posterior to the brain, and the injection of current (up to $\pm 10\text{ nA}$) did not influence the response. This is a strong indication that synaptic inputs to PI(2)5 are confined to the brain.

Visual inputs

PI(2)5 was phasotonically excited by switching on the illumination of the panorama (Fig. 3). The tonic response adapted beyond threshold after several seconds. The number of spikes varied with the position of the horizon. The larger the roll-deviation to the ipsilateral side (i.e. towards the axon) the larger was the response (Fig. 3). The adequate stimulus was illumination of the eye contralateral to the axon (i.e. ipsilateral to the main branches in the brain). Similarly, pitch-up deviations increased the illumination caused by light-on and thus the response of the neurone, whereas pitch-down had the opposite effect (data not shown). Light-off stimuli were only rarely followed by an action potential, and the latency exceeded that of the light-on response by more than 50 ms suggesting that it was caused by secondary inputs.

The response of PI(2)5 to visually simulated course deviations was *direction-specific*. Deviations in the preferred direction, i.e. roll and yaw to the side of the axon, and pitch-up, elicited bursts of action potentials (Fig. 4). Deviations in the antipreferred direction were only occasionally followed by one or several spikes, but on average this response did not exceed 10–15 % of that in the preferred direction (Fig. 5A,B; see also Fig. 7A). The response depended on the velocity and amplitude of the deviation, but because of variability (see final section of

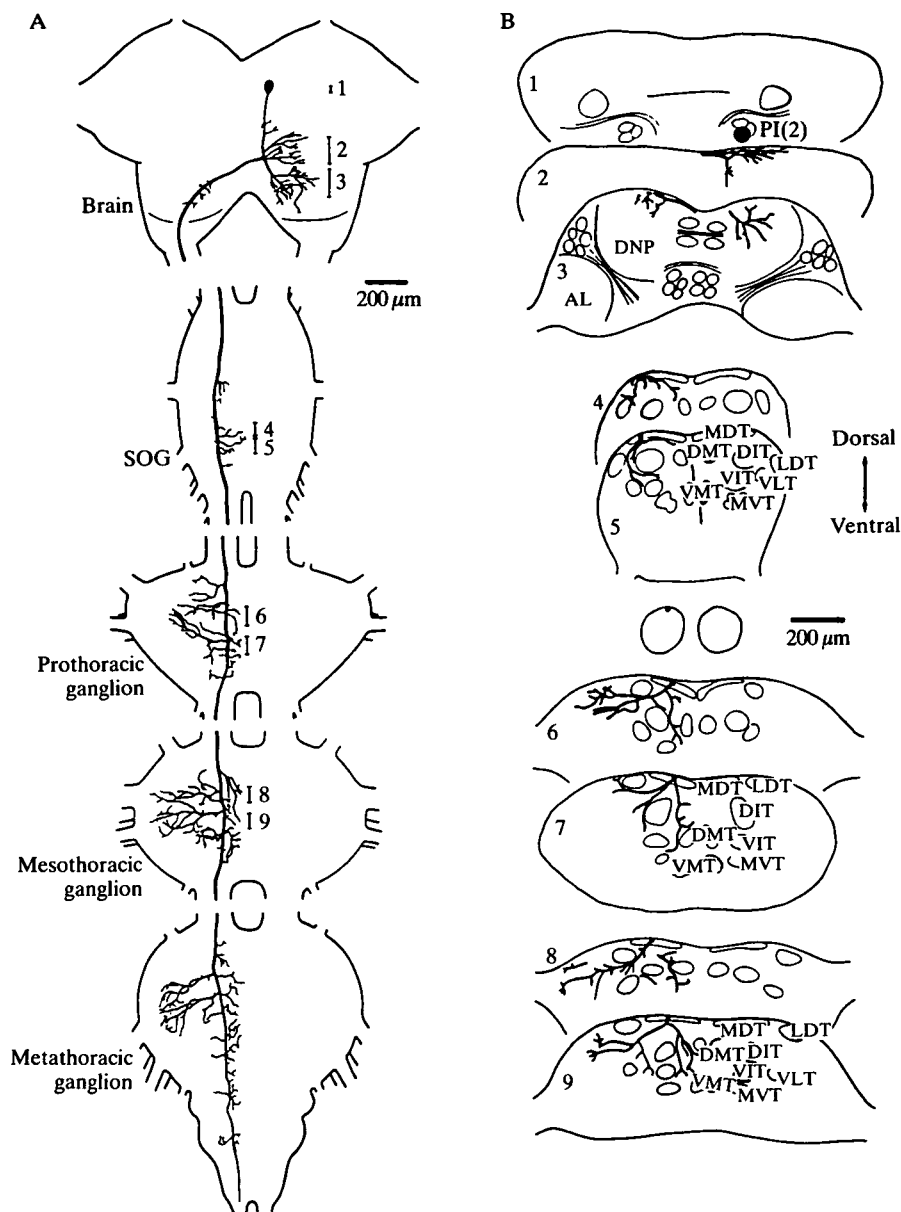


Fig. 2. (A) Dorsal view of the pars intercerebralis neurone PI(2)5 in the brain, the suboesophageal ganglion (SOG) and the pro-, meso- and metathoracic ganglia. By the terminology adopted here (see text) this is the left-hand PI(2)5. The term dorsal is used with respect to the neuraxis, i.e. compared with the intact animal the brain is tilted forward by about 90°. (B) Superimposed drawings of 2-4 adjacent vertical sections from the numbered regions. The projection fields in the metathoracic ganglion are similar to those in the pro- and mesothoracic ganglia. Tracts are designated after Tyrer & Gregory (1982). PI(2), pars intercerebralis medialis; DNP, dorsal neuropile; AL, antennal lobe; MDT, median dorsal tract; DMT, dorsal median tract; VMT, ventral median tract; LDT, lateral median tract; DIT, dorsal intermediate tract; VIT, ventral intermediate tract; MVT, median ventral tract; VLT, ventral lateral tract.

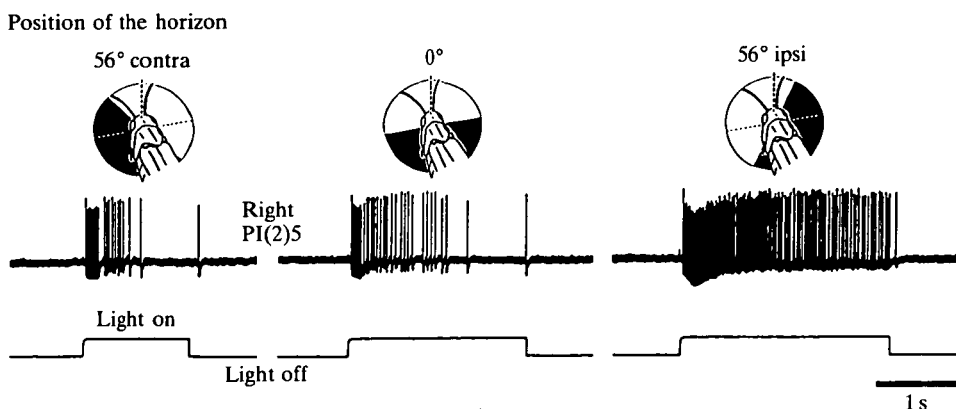


Fig. 3. Response of a right PI(2)5 to switching on and off the illumination of the panorama at different roll positions of the horizon (see insets). The terms ipsi- and contralateral are used with respect to the axon.

Results) a threshold could not be determined. Responses to simultaneous deviations around different axes were additive. For example, pairing roll and pitch (Fig. 5) increased the response when both deviations were in the preferred direction, and decreased the response when one deviation was in the antipreferred direction.

The responses to roll and pitch deviations was also *sector-specific*. This means that the number of spikes following deviations of identical amplitude depended on the sector of the visual field initially covered by the horizon. The more the horizon was oriented into the preferred direction, the larger was the response (Fig. 4A,C, dots in Fig. 9A), and in about 50% of the preparations a tonic component appeared. Sector specificity has also been described for DNI and DNC (Rowell & Reichert, 1986). In Fig. 4B the response to yaw deviations also seems to depend on the horizon position. However, this is regarded as artefact, because yaw moved the edges of the semihorizon into the visual field, which could thus interfere with the response, either directly or by changing alertness. This assumption is further supported by the observation that in most other individuals the response to yaw deviations was constant.

Visual inputs were confined to the compound eyes. Neither covering the ocelli nor selective stimulation with light pipes had any effect. Covering one or other eye revealed essential input from the contralateral eye and only weak excitation from the ipsilateral eye (Fig. 6A). The response to a deviation in the preferred direction was weaker if it followed soon after one in the antipreferred direction (Fig. 6B). This effect differed from habituation. The choice of the 100% level at a delay of 1.6 s was arbitrary and did not represent the maximum response obtainable. According to a second-order polynomial curve fit, this maximum appeared with a delay of 4–5 s, producing 125–130% of the response seen after 1.6 s.

The response to deviations could be due either to the detection of moving

objects or, during roll and pitch, to changed illumination of the contralateral eye (see Fig. 3). This was investigated by replacing the standard horizon with other visual patterns (Fig. 6C). A simple horizon reduced the stimulus to a single moving edge, but favoured light–dark changes, and a pattern consisting of randomly distributed dots excluded the latter. In both cases the response was significantly smaller than that to the standard horizon, which included both elements, suggesting that both kinds of input were combined. This conclusion was confirmed by turning the horizon upside-down, thus inverting the relationship between direction of movement and change of illumination (Fig. 6D). As could be predicted, PI(2)5 was now activated by deviations in both directions. Small moving objects only occasionally elicited spikes. PI(2)5 is therefore regarded as a specialized large-field movement detector.

No response was seen while rolling a white, unstructured hemisphere instead of the horizon. This proves that possible disturbing influences from moving parts of the lateral holder could be neglected during roll deviations. However, during pitch

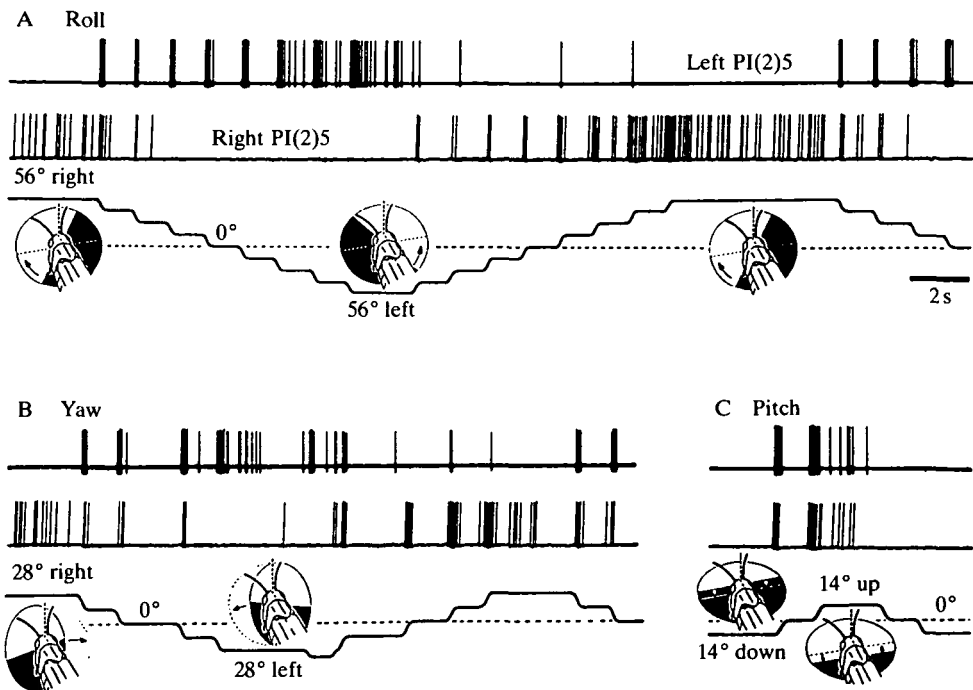


Fig. 4. Response of simultaneously recorded left and right PI(2)5 to deviations around the roll (A), yaw (B) and pitch (C) axis, respectively. Lower traces: the artificial horizon was moved in steps of 14° (maximum velocity 90°s^{-1}) between extreme positions. Dashed lines indicate the normal horizontal orientation (0°). The head was fixed in the normal position. Simulated deviations are designated as seen by the animal, e.g. movements of the horizon to the right are treated as deviations of the animal to the left (see insets). It should be noted that the response is direction-specific in all cases, and that it is sector-specific for roll and pitch deviations, i.e. the number of spikes depends on the sector of the visual field initially covered by the horizon.

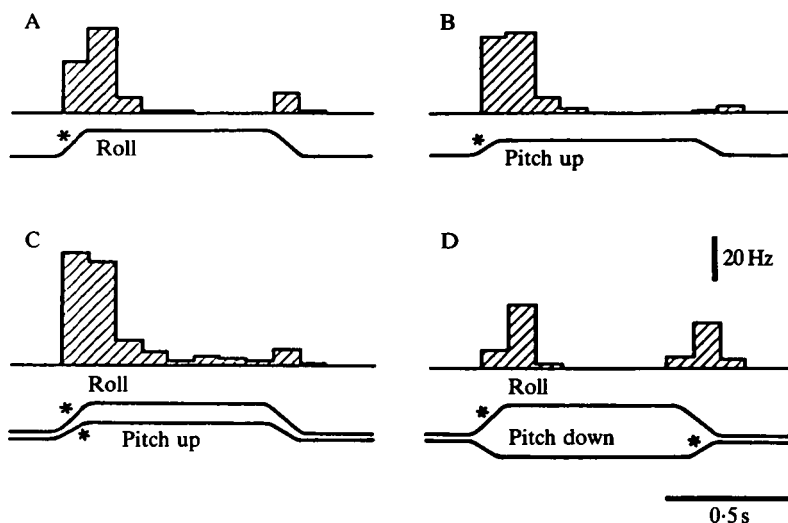


Fig. 5. Mean responses ($N = 20$) to trapezoid roll (A) or pitch (B) deviations of 28° and 14° , respectively, starting from the normal position. (C,D) Roll deviations were paired with pitch-up and pitch-down deviation, respectively. Asterisks mark movements in the preferred direction.

and yaw the edges of the hemisphere interfered with the image and could change the response. For this and other purely practical reasons (e.g. yaw and pitch movements were restricted by the experimental setup) the quantitative measurements presented in the following section are confined to roll.

Head movements

Proprioceptive inputs to PI(2)5 became apparent when comparing the response to horizon rolling with that to head rolling causing identical visual input. Head rolling changed the response in two ways (compare Fig. 7A,B). First, head movements in both directions were effective stimuli, thus direction-specificity was drastically reduced. The ratio between the response to movement in the antipreferred direction (open bars in Fig. 7B) and that to movement in the preferred direction (crosshatched bars in Fig. 7B) approached 1 (no direction-specificity) as the movement amplitude was reduced. Second, the response to head movements causing visually preferred input was larger than to identical visual input from horizon movements. The relative difference increased drastically at small movement amplitudes. Visually simulated deviations of less than 5° only occasionally caused a response whereas head rolling of only $1\text{--}2^\circ$ was usually followed by several spikes. As for visual inputs, no reliable threshold amplitude could be determined.

The inputs from neck receptors were experimentally separated from visual inputs in the following experiments. Movement of the head in the visually antipreferred direction in an unstructured panorama elicited a stronger response than movement in the visually preferred direction (Fig. 8, centre). The response to

head movements in the antipreferred direction was also stronger in an unstructured panorama than with the standard horizon (Fig. 8, left). This demonstrates an attenuating influence of horizon movement in the antipreferred direction. This could result from inhibition or removal of steady excitation by changed illumination (cf. Fig. 6C,D). In the dark the response was only weak (Fig. 8, right) and labile, and in almost 50 % of cases it failed completely. Thus the presence of light tonically increased the excitability from head movements. This conclusion was further substantiated by rolling head and horizon in parallel (thus excluding visual detection of movement), starting at different horizon positions (thus changing the overall illumination at the contralateral eye; columns in Fig. 9A). The change of

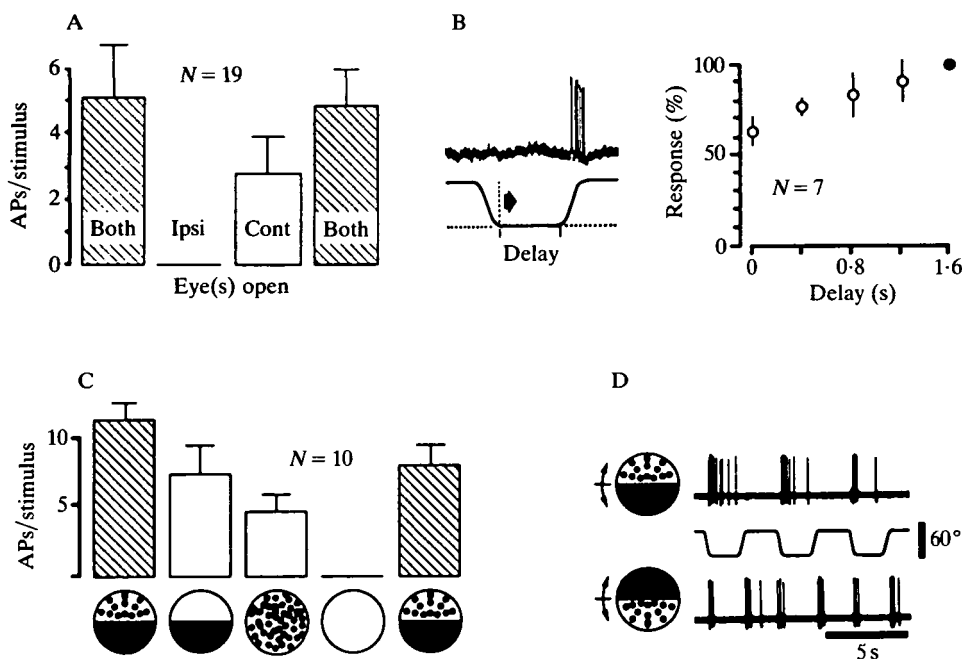


Fig. 6. Experiments to reveal the origin of visual input to PI(2)5. (A,C) Mean responses (error bars = 1 s.d.) to roll deviations of 28° in the preferred direction from representative experiments. Single measurements were made in blocks of N repetitions in the sequence represented by the left-to-right order of the columns. Crosshatched columns are controls; AP, action potential. (A) Only one eye open (ipsi- or contralateral with respect to the axon). (B) Responses to roll deviations (28°) in the preferred direction following movements in the antipreferred direction with various delays (dotted line = normal position of the horizon). Each dot represents 10 repetitions in seven different cells (error bars = 1 s.d.). Values are normalized for each cell with respect to the response at a delay of 1.6 s (solid circle). (C) Responses to deviations simulated with differently structured visual surrounds. In all cases the overall luminance was about 70 cd m^{-2} . (D) Responses to trapezoid roll deviations from 28° left to 28° right, the horizon being oriented normal or turned upside-down, thus inverting the relationship between direction of movement and change of the illumination during roll deviations.

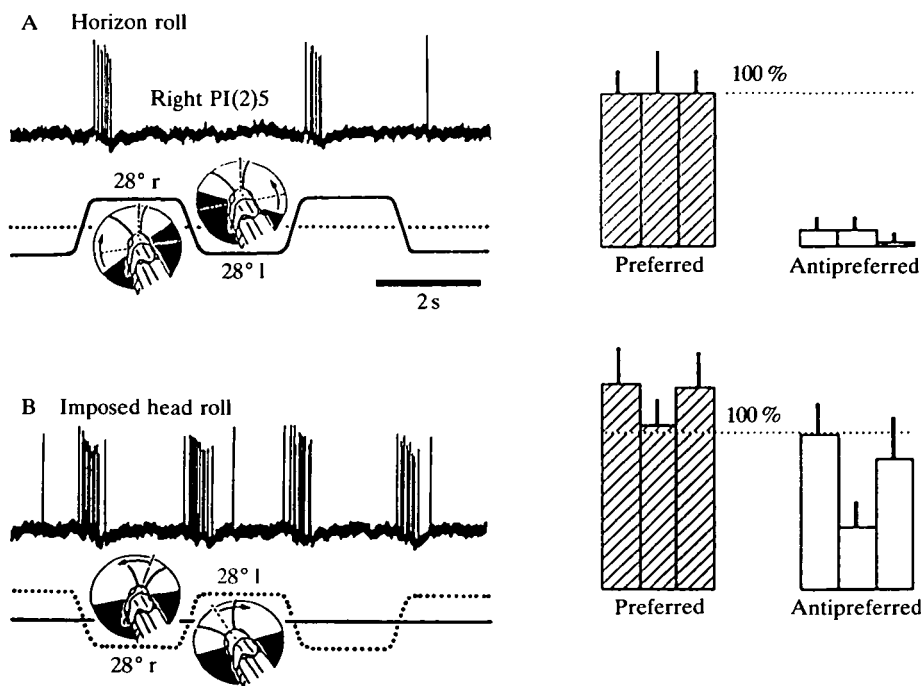


Fig. 7. Response of a right PI(2)5 to visually simulated roll deviations with the head fixed (A) or to imposed head rolling with the horizon fixed (B), both situations resulting in the same visual input. Continuous line, position of the horizon; dotted line, position of the head. Columns, mean responses (error bars = 1 s.d.; $N = 10$) in three different individuals, normalized with respect to the values for visually simulated deviations in the preferred direction (crosshatched columns in A).

the response was equivalent to that of visual sector specificity (dots in Fig. 9A; cf. Fig. 4A), suggesting the same underlying mechanism, i.e. tonic, subthreshold input signalling the overall illumination of the contralateral eye.

Combination of horizon and head movements

Tonic input by static head deflections

The response to identical deviations of the horizon was modulated by the position of the head with respect to the prothorax (Fig. 9B). Keeping the head statically rolled in the visually preferred direction resulted in an increased response, whereas keeping it rolled in the antipreferred direction resulted in a decreased response. This proves the existence of tonic, subthreshold input from neck receptors, changing linearly over 28° of head rolling to either side. This is the normal range of compensatory head rolling (Taylor, 1981a,b).

Locusts measure head position/movement using hairplates on the cervical sclerites and the myochordotonal organ of muscle 54 (Goodman, 1959, 1965; Shephard, 1974). Neurones from these organs enter the prothoracic ganglion *via* its lateral nerves. Severing these nerves reduced the modulating effect of head

position by more than 75 %. The remaining effect suggests that other receptors were important as well, most probably mechanoreceptors on the posterior of the head. In addition, this lesion generally reduced the response to horizon movements, indicating that tonic excitation had been removed.

Phasic input by compensatory head movements

In the experiments shown in Fig. 7 movements of the retinal image and head movements were in opposite directions. In contrast, compensatory head movements are in the same direction as the underlying deviations, thus minimizing slip of the retinal image (Fig. 10A). The delay between imposed deviations and compensatory head movements was 50–100 ms, depending on the illumination (see also Taylor, 1981a).

Compensatory head movements start 20–30 ms after steering reactions of wings, legs and abdomen (D. Robert, personal communication), but owing to the larger inertia of the body it can be assumed that during free flight the head is first realigned to the horizon (as seen in flies; Land, 1975). Thus a progressing deviation might already be counteracted both by steering and by compensatory head movement. This situation was simulated in the dissected preparation, by moving the horizon sinusoidally away from and back to the normal position (Fig. 10).

It turned out that the loss of visual input resulting from the compensatory head movement was balanced, and in most trials even overcompensated, by input from neck receptors. This was the case for active head rolling performed by the animal (Fig. 10C), and for imposed head rolling (Fig. 10D,E second column). Imposed head rolling of more than 8° could increase the response by more than 100 % above the control value (e.g. Fig. 10E, second column). In dissected, non-flying

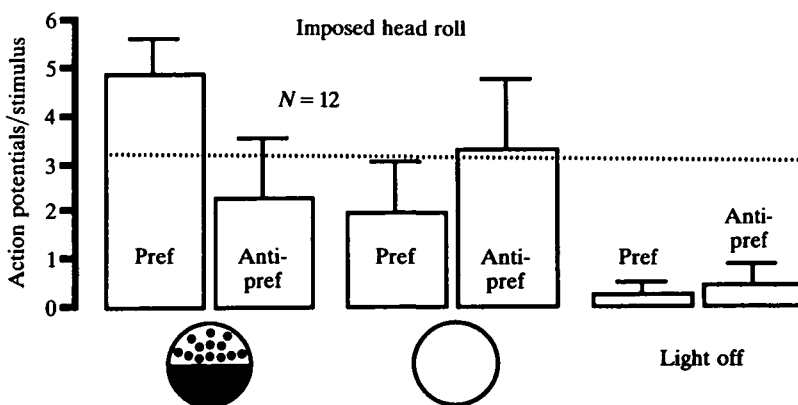


Fig. 8. Mean responses (error bars = 1 s.d.) to trapezoid head rolling of $\pm 14^\circ$ (same course as in Fig. 7) in the visually preferred and antipreferred direction, respectively. The animal faced the standard horizon, or an illuminated but unstructured panorama, or the lights were switched off (see insects). The dotted line is interpolated between control values recorded before and after the experiment. It represents the response to horizon movements in the preferred direction causing identical visual inputs like head rolling.

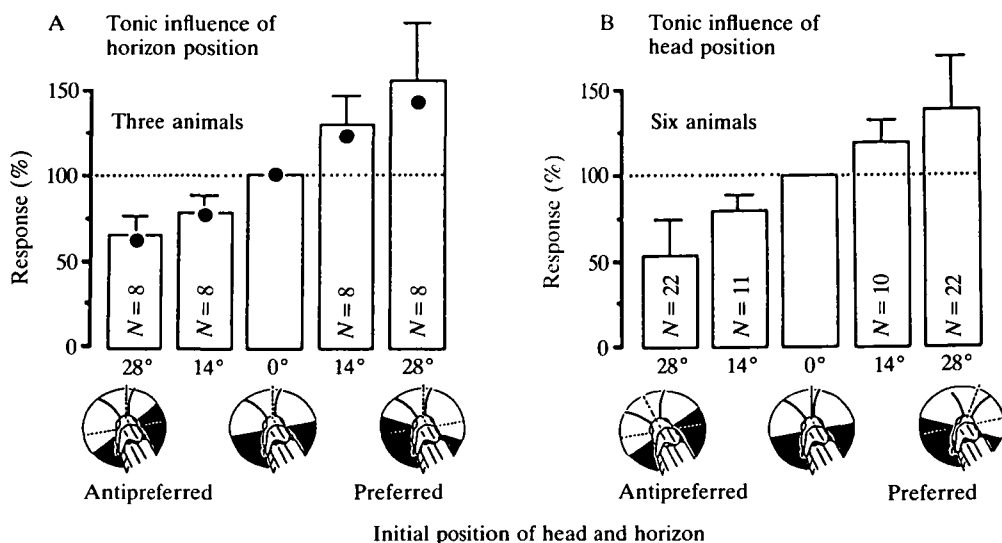


Fig. 9. (A) Dots, normalized responses to steplike horizon rolling in the visually preferred direction at various initial orientations of the horizon (see Fig. 4A). Columns, normalized responses to head rolling from 14° left to 14° right at various initial orientations of the horizon. Visual input was excluded by rolling the horizon in parallel with the head, starting from the initial situation as indicated by the insets. (B) Normalized responses to horizon rolling of 28° in the visually preferred direction at different head positions with respect to the prothorax. Before each experiment the horizon was aligned to the head, so that the visual situation was the same in all cases (see insets). (A,B) Values represent the mean number of action potentials in the left PI(2)5 during the first 500 ms after the start of the response (error bars = 1 s.d.). *N* is the number of single experiments, each one representing 10 successive stimuli.

locusts, active compensatory head rolling was mostly restricted to a narrow range of relatively small amplitudes (Fig. 10C; mean: $4.8 \pm 1.2^\circ$, $N = 80$ from eight animals). Nevertheless, the response increased by $26 \pm 14\%$. On average this increase was equivalent to that obtained by imposed head rolling of the same amplitude, suggesting that during passive and active head movement PI(2)5 was excited *via* neck receptors and not by central connections, as might be expected for active head rolling. The enhancing effect of compensatory head roll is further demonstrated in Fig. 10E. Visual input was identical in the second and third columns but because of missing proprioceptive input in the third column the response was drastically reduced.

In Fig. 10D it is conspicuous that the response was only weak while the head returned to the normal position, although Figs 7B and 8 show that head rolling in either direction was an effective stimulus. This discrepancy results from suppression of the response due to the parallel horizon movement in the antipreferred direction (cf. Fig. 8 left and middle) and from the sinusoidal head movements (in contrast to trapezoid movements in Figs 7, 8) leading to the same effect as shown

for horizon movements in Fig. 6B: the response to head rolling is reduced when immediately following head rolling in the opposite direction.

Frontal wind and activity during flight

Some DNs are excited by frontal wind, whether the animal shows flight activity or not (e.g. Bacon & Tyrer, 1978; Rowell & Reichert, 1986). In contrast PI(2)5 was tonically inhibited by frontal wind, as shown by a decreased response to deviations or head movements (Fig. 11A). The amount of inhibition depended on the wind velocity (Fig. 11B). No directional effect was found. Wind receptors are excited during flight, and PI(2)5 therefore seems to be disqualified from being involved in flight steering. However, flight activity itself caused tonic excitation of central origin (Fig. 12A,Bii) which overrode wind inhibition, so that the response to

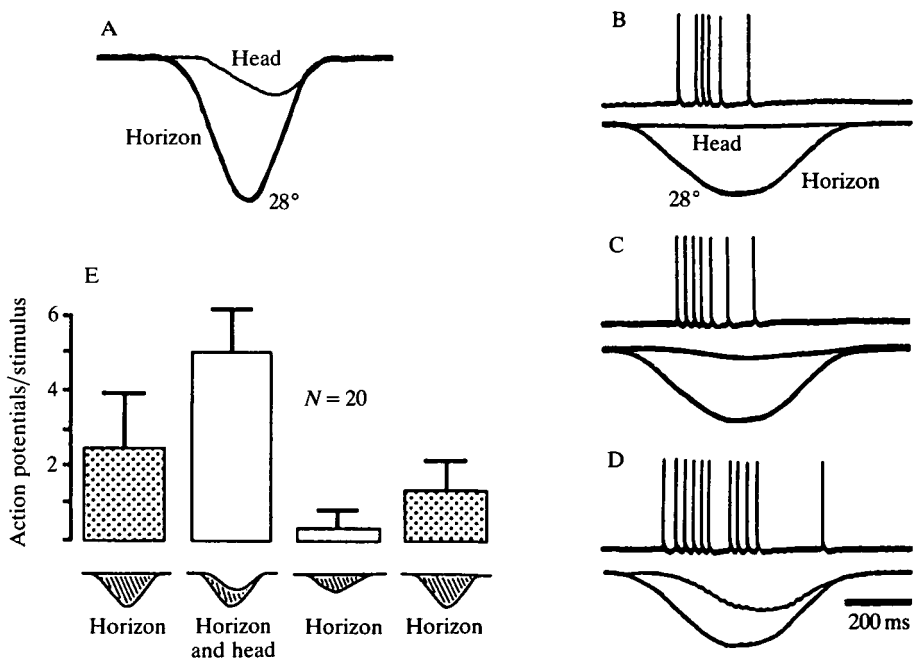


Fig. 10. (A) Sinusoidal rolling of the horizon simulated the situation during free flight: horizon movement away from the normal position to simulate course deviation; horizon movement back to the normal position to simulate a steering response. After a delay, retinal slip is reduced by compensatory head movement (intact, non-flying locust). (B–D) Responses of PI(2)5 to the same horizon movement as in (A) (dissected animal): (B) while head rolling was prevented, (C) while the locust actively performed compensatory head rolling (because of the dissection the amplitude was reduced to less than 5°), and (D) while compensatory head rolling of about 14° was imposed under servocontrol. (E) Stippled columns, control values from recordings with head fixed as in (B). Second column, horizon and head rolling as in (D). Third column, net visual input identical to that in the preceding experiment but head movements prevented. Crosshatched areas indicate the visual input.

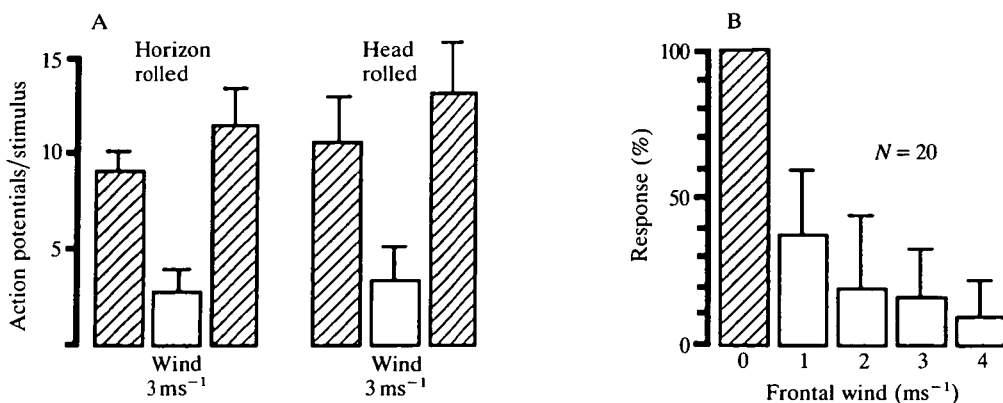


Fig. 11. Tonic inhibition of PI(2)5 by frontal wind. (A) Mean responses ($N = 20$, error bars = 1 s.d.) to roll movement of horizon or head ($\pm 28^\circ$) without wind (crosshatched columns) or with a frontal wind of 3 m s^{-1} . (B) Normalized mean responses to roll movements of the horizon ($\pm 28^\circ$) at different velocities of frontal wind.

deviations was even larger than at rest (Fig. 12B,C). During the initial phase of flight PI(2)5 spiked tonically (20–80 Hz), but during longer sequences (>30 s) the flight-dependent excitation often adapted below threshold. Nevertheless, the phasic responses to deviations were larger than in non-flying animals. When flight activity ceased, the inhibition predominated again until the wind was switched off (Fig. 12Biii,iv). The excitation was weakly modulated in the flight rhythm (Fig. 12D). It should be noted that excitation commenced about 160 ms before the activity in M112 (metathoracic, indirect wing depressor), the only wing muscle with intact innervation.

Variability of the response

The response to equal stimuli was variable, even when presented to the same individual at different times. This was due to habituation and also to internal influences of unknown origin. The latter could occur in quiet animals without recognizable external input, and was expressed as: sudden activity; or modulation or cessation of hitherto constant responses to deviation; or both of these phenomena. The course of habituation was approximately exponential, reaching a plateau value, similar to that of other visual neurones (e.g. descending contralateral movement detector, DCMD: Rowell, 1974). The height of the plateau depended on the interstimulus interval. Complete habituation occurred only at repetition rates above 1–2 Hz. PI(2)5 was dishabituated to various degrees by different sensory stimuli such as wind, touching various parts of the body, and light-on/off, but also after flight or other active movements (see increased response after wind in Fig. 11A).

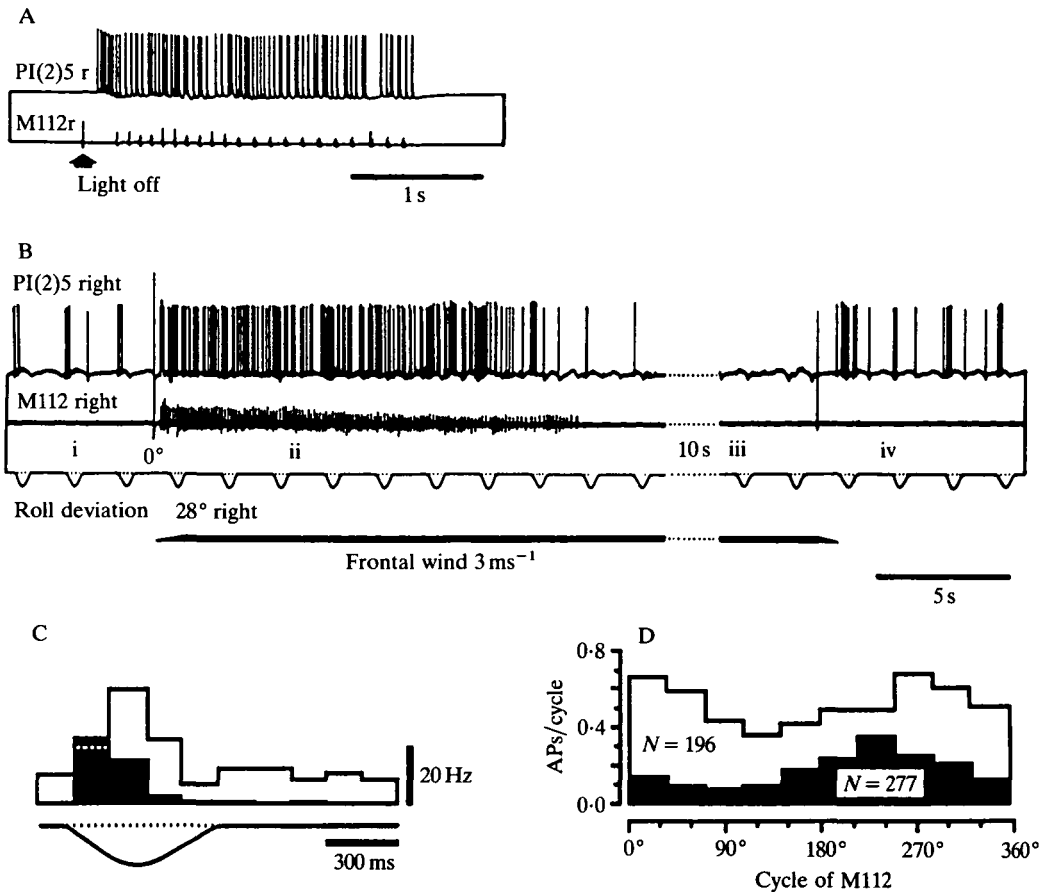


Fig. 12. (A) Tonic excitation of PI(2)5 during flight activity without wind, triggered by a light-off stimulus (light-off without flight activity does not cause excitation; see Fig. 3). Flight activity is indicated by rhythmic firing of muscle 112 (indirect wing depressor). (B) Response of a right PI(2)5 to visually simulated roll deviations to the right, (i) at rest, (ii) during fictive flight with maintained frontal wind, (iii) with frontal wind after the cessation of flight, and (iv) at rest again. (C) Mean response to the simulated deviations shown in (B), before (black histogram; $N = 6$) and during fictive flight (clear histogram; $N = 8$). (D) The excitation during flight was weakly modulated in the flight rhythm. Values from two animals are shown. 0° represents the first M112 spike of a cycle, 360° the first spike of the following cycle.

Discussion

Criteria for identification

PI(2)5 can be identified physiologically as follows: light-on, pitch-up and ipsilateral roll or yaw deviations (i.e. towards the recorded axon) elicit bursts of spikes, sometimes accompanied by a tonic component which adapts after a few seconds (Figs 3, 4). Under the conditions used here, the response does not habituate completely at repetition rates below 1 Hz. Flight activity causes tonic

excitation (Fig. 12) and frontal wind causes tonic inhibition (Fig. 11). Further criteria not necessary for identification are that head movements in either direction evoke a burst of spikes (Figs 7B, 8), and that compensatory head movements reinforce the visual response to deviations (Fig. 10). Neurones fulfilling these criteria are morphologically compatible with PI(2)5 of Williams (1975), and are thus considered to be identical. Owing to its large size PI(2)5 appears to be unique and not one of several morphologically similar neurones.

Processing and convergence of inputs

PI(2)5 is the first DN of locusts known to receive convergent inputs from exteroceptors on the head, proprioceptors of the neck, and from the flight motor. Movements, and the position of horizon and head are coded by phasic suprathreshold input and by tonic subthreshold input, respectively (Figs 4, 7, 9, 12). Single mechanoreceptive hairs on the neck display a similar response pattern to steplike deflection: a burst of action potentials (up to 300 Hz) adapts within 1 s to a tonic level of 40–50 Hz (Haskell, 1959). It is assumed that the tonic inputs from these receptors to PI(2)5 (Fig. 9B) are exclusively excitatory, the head position being represented by the sum of their activity. Drastic reduction of responsiveness, seen after denervation of the prothoracic ganglion, supports this view. Input regions seemed to be confined to the brain, but none of the known and presumed neck receptors projects into the brain (Bräunig *et al.* 1983; Anderson, 1985). Hence, intercalated, ascending neurones must be postulated.

The processing of visual inputs is more complex. Position and movements of the horizon around the roll and pitch axis are detected as intensity and change, respectively, of the overall illumination at the eye contralateral to the axon (Figs 3, 6D, 8, 9A). Movement detection in ocellar neurones functions similarly (Simmons, 1982; Rowell & Reichert, 1986), but ocellar neurones are excited by light-off, whereas PI(2)5, which receives no ocellar input, is excited by light-on. Deviations are further recognized by the detection of moving cues in the panorama (Fig. 6C). Both mechanisms work synergistically during roll and pitch deviations but during yaw the overall illumination does not change and deviation detection must be based entirely on the second mechanism. This also excludes sector specificity with respect to the yaw axis.

The combination of phasic and tonic responses from eyes and neck receptors elegantly solves some problems associated with corrective flight steering. (1) Sector specificity, which is essential for corrective steering around the roll and pitch axes (see below), comes about by superimposition of phasic visual input onto subthreshold tonic visual input (Fig. 4, 9A). (2) The reduction of visual input about deviations by compensatory head movements is balanced, in most cases even overcompensated, by phasic input from neck receptors (Fig. 10C–E). (3) The tonic, modulatory effects of horizon and head position are of similar strength. Thus, if the body is aligned to the horizon, but the head is rolled with respect to it, e.g. towards the visually preferred direction (right column in Fig. 9A), then the head is also rolled with respect to the body but towards the

antipreferred direction (left column in Fig. 9B). The amplifying influence of the mismatch between head and horizon is balanced by the attenuating influence of the mismatch between head and body. As a consequence, sector specificity in PI(2)5 relates to the body and not to the head.

The tonic inhibition by frontal wind (Fig. 11), and the tonic excitation during flight activity (Fig. 12), do not contain directional information, but they influence the general excitability of PI(2)5. The function of wind inhibition is unknown. The excitation during flight enhances the spiking response to deviations, and eventually makes it more tonic. This excitation must be of central origin, as it starts some 160 ms before activity in M112 (the only wing muscle with intact innervation), and as all proprioceptors of the wings were denervated in the respective experiment. At the onset of flight activity, its start coincides with the initial subthreshold activity in wing motoneurons (Robertson & Pearson, 1982; Hedwig & Pearson, 1984). The weak, cyclic modulation (Fig. 12D) might be centrally produced, but it could also reflect sensory feedback that was reporting rhythmic contractions in neck and/or other muscles of the neck and prothorax.

One could speculate that further sensory inputs converge onto PI(2)5 but, except for direction-specific excitation by leg movements (Kien & Altman, 1984), evidence for this is only weak. PI(2)5 has been shown to be frequently excited when various parts of the body were touched, but no location could be specified at which the response was sharply correlated with the stimulus (K. Hensler, unpublished observations). This suggests secondary excitation by the active movements normally following a touch. Wind blown onto the cerci from various directions often increased the response by 20–30 %, but this effect was labile and may have been due to dishabituation or changed alertness (K. Hensler, unpublished observations).

In summary, the number of spikes following horizon and/or head movements is determined by the sum of various tonic inputs. However, the excitability depends also on the state of habituation/dishabituation, and it may vary considerably due to unforeseeable internal events of unknown origin, which have also been described for other DNs (Rowell & Reichert, 1986). Thus a locust may respond to a stimulus or ignore it, an important prerequisite for behavioural plasticity.

Comparison with other DNs in locusts and other insects

Four other locust DNs have been described in detail (TCG, DNI, DNC and DNM, see Introduction). TCG is influenced neither by movements of the horizon nor by neck receptors (K. Hensler, unpublished observations). DNC appears to receive inputs from neck receptors in the same way as PI(2)5, although these inputs are weaker than the visual input (K. Hensler, in preparation). DNI and DNM require further investigation. Other DNs, not yet completely identified morphologically, also respond to movements of horizon and head, as shown for PI(2)5 (K. Hensler, in preparation).

During roll and yaw deviations, PI(2)5 responds like DNI of the same connective. However, during pitch deviations the preferred direction of PI(2)5 is

upwards, whereas the preferred direction of DNI, DNM and DNC is downwards. Further differences are that TCG, DNI, DNC and DNM are excited by frontal wind, whereas PI(2)5 is inhibited. Except for TCG, none of the other three DNs is influenced by flight activity (data for DNI, DNC and DNM from Rowell & Reichert, 1986; data for TCG from Bacon & Möhl, 1983).

All known DNs code for different deviations or combinations of deviations which are perceived using inputs from various sensory sources. This is reflected in their different brain morphologies. In contrast, the thoracic structures are very similar, indicating their common function, the control of neck and wing muscles during flight steering. This function has been demonstrated directly by electrically stimulating single DNs in intact animals (Möhl & Bacon, 1983; K. Hensler & C. H. F. Rowell, in preparation) and indirectly by demonstrating connections to flight motoneurons and premotor interneurons (Tyrer, 1981; Simmons, 1980; Reichert & Rowell, 1985).

DNs have also been described for a number of other insect species (discussed by Rowell & Reichert, 1986), but convergent inputs from neck receptors have been reported only for visually driven self-movement detectors in the dragonfly (Olberg, 1981). Although these units were not identified morphologically, it is reasonable to assume that they are equivalent to locust DNs.

A model of corrective flight steering

The phasic bursts signalling course deviations in PI(2)5 and other DNs (Rowell & Reichert, 1986) provide the locust with an impulse of corrective torque. The resulting steering response causes a movement of the retinal image in the opposite direction, thus exciting DNs with the opposite preferred direction. Activity in these DNs would balance the steering response and thus prevent correction if it were not suppressed. This suppression comes about by temporary reduction of the excitability following antipreferred deviations (Fig. 6B), and by sector specificity, leading to stronger responses when the horizon moves away from the normal position than when it moves towards the normal position. Sector specificity is a common feature of DNs (Rowell & Reichert, 1986; K. Hensler, in preparation). It is, however, only effective around the roll and pitch axis, so that yaw correction must involve orientation towards objects (Baker, 1979; Dugard, 1967).

Another important aspect of sector specificity is that it contains information about the absolute position of the horizon in the visual field. If steering were to be based simply on the detection of movement, even small mistakes in a series of correction manoeuvres could accumulate and finally lead to a crash. Due to sector specificity in DNs acting in opposite directions (e.g. contralateral homologous), the flight position is stabilized around that point where the activity in all DNs with the same preferred direction balances activity in all DNs with the opposite preferred direction. This position is reached when the horizon is oriented horizontally in the visual field, the bright part being above. Thus sector specificity leads to a 'dorsal light response' (e.g. Wehner, 1981).

As long as head and body are aligned with each other, mere exteroceptive

deviation detection is sufficient to maintain the optimal orientation of the body (i.e. the aerodynamic organs) with respect to the horizon (which is a good indicator for the direction of gravity). However, during compensatory (and other) head movements the relative orientation of head and body is changed, so that exteroceptors on the head can neither detect a remaining mismatch between body and outer world nor measure the amount of subsequent deviations. In PI(2)5 these problems are elegantly solved by the convergence of tonic, subthreshold inputs from eyes and neck receptors, which couple sector specificity to the orientation of the body (see above).

Implications for further studies

As demonstrated here for PI(2)5 and elsewhere for other DNs (Rowell & Reichert, 1986), sensory interneurons, although multimodal, might not respond to inputs from a single modality unless this is presented in the right context or in combination with other stimuli. For example, PI(2)5 is only occasionally excited by head rolling in the dark, but in combination with light or horizon movements head rolling is an effective stimulus (Fig. 8). This finding could explain the weak steering reactions caused by forced head movements in the dark (Taylor, 1981*b*), which might otherwise be interpreted as indicating a subordinate function of neck receptors in flight steering.

It is a general principle that neurones or neural systems respond selectively to specific constellations and combinations of stimuli as they occur during normal life (Wehner, 1987). As a practical consequence, investigators should mimic the respective behavioural situation as closely as possible. The simplest example of the effectiveness of such an approach is represented by the observation that a relatively rich visual world resulted in a significantly better response of PI(2)5 than a simple horizon or a dot pattern (Fig. 6C).

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