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Original article

Antennal reflexes in the honeybee: tools for studying the nervous system

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Summary — Honeybees respond with antennal reflexes to stimuli of different modalities when kept in the restricted conditions of a laboratory experiment. The animals react with direction-specific antennal responses to vertically moving stripe patterns. Stripe patterns with velocities between 25°/s and 60°/s evoke maximum responses. The minimum pattern size is 55° x 35° relative to the bee eye. Unilateral stimulation with the pattern elicits ipsilateral antennal responses. Bees respond to olfactory stimuli with directed scanning movements of both antennae. The magnitude of the response depends on the logarithm of the odour concentration. Different classes of odours evoke different responses. Bees touch objects briefly and frequently with their antennae. The median contact duration is < 10 ms; the frequency of contact ranges from < 3/min to > 300/min. The mechanical antennal response shows a high degree of plasticity. Bees appear to learn the position of an object and scan the area and contours of the object for at least 2 min after it has been removed. The antennal reflexes of the bee serve as tools for studying signal processing, neuromodulation, sensitisation and learning in different sensory systems.

***Apis mellifera* / antennal reflex / plasticity / neurobiology**

INTRODUCTION

Many neurobiological studies in the honeybee aim at understanding the underlying neural mechanisms of behaviour (Erber *et al*, 1991; Fonta *et al*, 1991; Menzel *et al*, 1991). Unfortunately, we cannot register the neural activity in the bee during its normal behaviour in the hive or during a foraging flight. We are forced to study behaviour under very restrained laboratory

situations. The interpretation of neuroethological analyses in the bee critically depends on behavioural observations which can be made under the restrained conditions of electrophysiology. In an ideal experiment, behaviour occurs during the electrophysiological recording. In many cases we cannot reach this ideal goal and have to rely on behavioural studies which are made under conditions identical to the physiological experiment. Due to the enor-

mous variety of bee activities, we can only carry out successful neurobiological analyses if we are able to study a number of different activities under laboratory conditions.

The proboscis reflex in response to sugar water stimulation of the antennae has been used for analyses of olfactory conditioning, studies of habituation and as a behavioural measure of modulatory effects of putative neurotransmitters in the brain (Kuwabara, 1957; Erber *et al*, 1980; Mercer and Menzel, 1982; Macmillan and Mercer, 1987; Menzel *et al*, 1988; Braun and Bicker, 1992). This reflex is extremely useful for the study of associative and non-associative olfactory learning (Menzel, 1983). It can also serve as a tool for studying the perception of gustatory stimuli by the antennae.

We have studied antennal reflexes in the bee because the antennae display specific responses to different modalities without conditioning (Kloppenburg *et al*, 1989). Bees show antennal reactions to visual, olfactory, gustatory and mechanical stimuli. The complex antennal movements are controlled by 4 muscles in the head which move the scape and 2 antagonistically acting muscles in the scape which move the pedicel and the flagellum. The 4 muscles moving the scape are innervated by 9 motoneurons which have their dendritic projections in the dorsal lobe where they overlap with the 6 motoneurons of the muscle system within the scape (Ellerkmann *et al*, 1989; Kloppenburg, 1990; Kloppenburg *et al*, 1990b; Pribbenow, 1990). Neuroanatomical studies have shown that afferents of proprioceptors at the antennal joints and of the Johnston organ project into the dorsal lobe (Maronde, 1990, 1991; Pribbenow, personal observation). There is good evidence that mechanoreceptors of the flagellum also project into the ipsilateral dorsal lobe. There, they overlap considerably with the dendritic ar-

borisations of the antennal motoneurons (Kloppenburg, 1990). Another group of cells projecting into the dorsal lobe are higher-order visual interneurons from the third optic ganglion, the lobula (Maronde, 1991). The dorsal lobe represents the multimodal sensomotor neuropile of the antennae.

The antennal sensomotor system has been well studied. Its already defined network of sensory inputs from both the antennae and the visual system, together with the knowledge of the output motoneurons, offer a very useful approach to neuroethological studies in the bee. It is the aim of this paper to summarise the antennal responses to visual, olfactory and mechanical stimuli. The same motor output can be used to study the plasticity of signal processing in different sensory systems and to analyse the effects of neuromodulators in the nervous system of the bee.

MATERIAL AND METHODS

Honeybees were caught at the hive entrance, immobilized by chilling in the refrigerator at 5 °C and then harnessed in tubes for the experiments. Different methods were used to quantify the antennal responses.

Visual stimulation

In the first series of experiments visual antennal reflexes were analysed. Antennal responses were elicited by presenting moving stripe patterns to one or both compound eyes of the bee. The antennal responses were evaluated with an optoelectronic device. A single bee was fixed with its holder in a vertical position. The bee's head was illuminated from below with red light from a light guide. The contours of the bee head and the antennae were projected through a dissecting microscope to a ground glass disc mounted above one ocular. Eight phototransistors on the ground glass registered the movement of the antennae. The signals of the photo-

detectors were fed *via* an interface to a micro-computer which calculated the mean antennal angle for each antenna (fig 1A; for further details see Erber *et al*, 1991).

The antennal angle for each antenna is defined as the angle relative to the midline of the head (fig 1A). The 2 regular black and white patterns were presented at varying distances and velocities to the compound eyes. The stripe width was 1 cm. The pattern size was calculated as the visual angle relative to the bee's eye, its velocity as the angular velocity (degrees/s). The patterns were illuminated by white light through 2 light guides (reflected intensity 20–50 $\mu\text{W}/\text{cm}^2$) and driven by DC motors. The velocity, direction of movement and time of stimulation of the 2 patterns were independently controlled by an electronic timer.

The movement of the stripe pattern always lasted 10 s. Antennal angles were calculated for each antenna before stimulation (spontaneous angle), and during and after the stimulus. The antennal angles were first calculated as absolute angles. For many experiments the angular difference of the responses for 2 different directions of pattern movement was used as a measure of direction specificity. This "direction-specific antennal response" (DAR) can be used to standardize different experimental series (fig 1A; Erber *et al*, 1991).

Olfactory stimulation

Antennal responses to olfactory stimuli were measured with a simplified version of the optoelectronic device used for the visual experiments. Two phototransistors on the ground glass registered the frequency of antennal movements towards the midline of the head (for methodological details see Erber and Schildberger, 1980). The phototransistors were arranged on the ground glass at positions which were passed by the antennae when moving in the ventral direction. The antennae display rapid scanning movements towards the source of olfactory stimulation. Since the olfactory stimuli in our experiments were always presented from the ventral side, the antennae moved ventrally, thus frequently passing the 2 phototransistors (fig 1B). Therefore, the direction of antennal responses was indicated by the frequency of antennal passages.

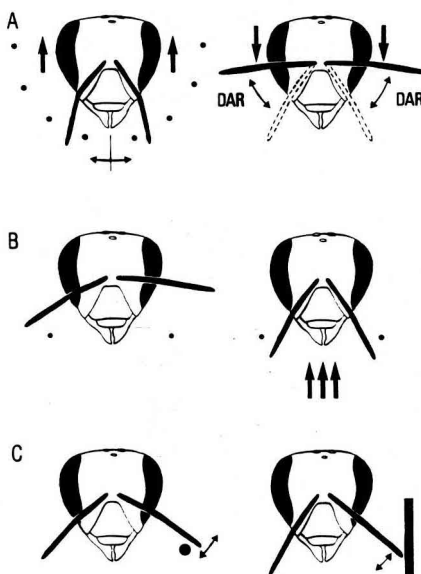


Fig 1. Schematic drawing of antennal responses and their registration. **A:** The visual antennal reflex: when the stripe pattern moves upward (arrows) relative to the bee, the antennae move downward. Downward pattern movement leads to upward antennal movement. The antennal angle of each antenna is measured relative to the midline of the head (left figure). The direction-specific antennal response (DAR) is the mean angular difference of the position of the antenna between upward and downward pattern movement (right figure). Antennal responses are measured by the indicated phototransistors on a ground glass. **B:** The olfactory reflex: a bee moves its antennae spontaneously around (left figure). During an olfactory stimulus, which is presented in an upward airstream (right figure; arrows), the bee moves the antennae downward in the direction of the stimulus source. Two phototransistors register the frequency of antennal passages; the relative response (passing frequency during a stimulation/spontaneous frequency) is used as the behavioural measure. **C:** The mechanical antennal response: a bee scans a wire (left figure) or a small metal plate (right figure) by rapidly touching it for median durations of < 10 ms. The antennal contacts are registered by connecting the objects to the input of an open high-gain amplifier while the insect is grounded.

Olfactory stimuli were applied by pumping an airstream (pressure 0.2 bar) through a glass tube containing a filter paper soaked in 50 μ l of a defined concentration of an odour. Tested odours were: paraffin oil (control), isoamyl acetate, citral, geraniol, caprylic acid (all from Sigma Chemie GmbH). An individual tube was used for each odour and each concentration. The airstream was directed through a nozzle ventrally towards the bee (fig 1B). The stimulation programme was controlled by an electronic timer. The stimulus sequence was chosen at random. Antennal passages were evaluated online by a computer. Both antennae's responses were measured over 15 s before the stimulus (spontaneous response), over 15 s during and 15 s after it. Their values were determined by calculating the passing frequency during and after a stimulus relative to the spontaneous frequency before stimulation. Changes of relative responses were only observed during the stimulus, not after. Relative response values > 1 indicate that the antennae moved towards the stimulus source.

Mechanical stimulation

Responses of a single antenna to mechanical stimuli were measured with an electronic device. An object, either a silver wire or a small silver plate, was connected to the input of an open differential high-gain amplifier (fig 1C). Contacts of the antenna with the object could be registered as rapid voltage changes whenever the antenna of the grounded animal touched the object. The signals were registered by a transient recorder and stored by computer. Both touching frequency and duration were analysed.

Behaviour was also registered by video recordings. Data were evaluated by frame-to-frame analysis using the still mode of the video recorder.

RESULTS

Visual antennal responses

Free-flying honeybees show specific antennal movements when landing in a visu-

ally structured environment (Erber, 1984). During flight the antennae are held in an upright position. Shortly before landing and only when approaching the landing platform from above are the antennae moved downward, thus scanning the landing platform. Very similar responses can be elicited in the laboratory by using upward-moving stripe patterns.

When a stripe pattern presented to both compound eyes moves upward relative to the bee, the antennae move downward. Opposite pattern movement leads to upward-moving antennae. The response shows short latencies. Figure 2A summarises the antennal responses to different directions of pattern movement. The antennal angles for the 2 vertical directions of pattern movement (fig 2A I) differ significantly ($p < 0.01$; 2-sided Wilcoxon test). The mean antennal angles during upward pattern movement also differ significantly from the spontaneous antennal angle before stimulation ($p < 0.01$, 2-sided Wilcoxon test). Vertical pattern movement therefore results in direction-specific antennal responses. The mean angular difference between the responses to the 2 stimulus directions, the direction-specific antennal response (DAR), is a measure of the directionality of the reflex. The DAR is $\approx 20^\circ$ for the experiment in figure 2A I.

Pattern movement along the body axis (longitudinal plane of movement) leads to antennal angles which are smaller than the spontaneous antennal angle (fig 2A II). The mean antennal angle for back-to-front movement of the pattern differs from the spontaneous angle ($p < 0.05$, 2-sided Wilcoxon test). Yet no direction specific response was found.

Movements of the striped patterns in different diagonal-vertical directions lead to antennal responses similar to those during vertical stimulation (fig 3 III, IV). The angular responses for the upward and down-

ward directions differ significantly in both experiments ($p < 0.01$, 2-sided Wilcoxon test). Similar to vertical pattern movement, the responses for the upward direction during diagonal pattern movement differ from the mean spontaneous antennal angle ($p < 0.025$, 2-sided Wilcoxon test). Honeybees respond with direction-specific antennal responses during upward and downward pattern movement. The DAR is highest for vertical movements.

However, it was not clear whether the antennal response is controlled by both eyes. Therefore, a series of experiments was designed to test different movement stimuli presented to one or both eyes. A "roll" stimulus, consisting of an upward-

moving pattern on one side and a downward-moving pattern on the other side, leads to significant direction specific responses of the 2 antennae (fig 2B I; $p < 0.05$, 2-sided Wilcoxon test). Each antenna responds according to the stimulus direction presented to the ipsilateral eye, thus suggesting that each eye governs the response of its side's antenna.

This hypothesis can be tested by stimulating only one compound eye and evaluating the antennal responses of each side separately (fig 2B II, III). The results demonstrate that the antenna on the stimulated side exhibits a significant direction-specific response (fig 2B II; $p < 0.025$, 2-sided Wilcoxon test), while the contralateral antenna on the unstimulated side does not display a direction-specific response (fig 2B III). This experimental series has shown that visual antennal responses are indeed governed by the ipsilateral compound eye.

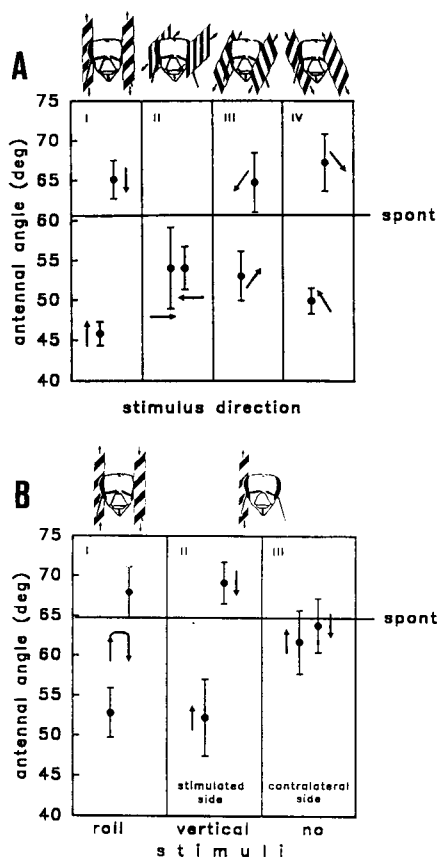


Fig 2. The visual antennal responses to moving stripe patterns: the ordinates show the mean absolute antennal angles (\pm SEM) for different experimental groups. **A:** The antennal responses to 2 stripe patterns moving in the same direction for both compound eyes. Pattern velocity was $48^\circ/\text{s}$, pattern size was $58.1^\circ \times 31^\circ$; the direction of movement is indicated by the drawings above the respective columns; 9 bees were tested in each group. The difference between opposite responses is the direction-specific antennal response (DAR). **I.** Responses to either upward or downward pattern movement (arrows). **II.** Responses to longitudinal movement (arrows). **III, IV.** Responses to diagonal pattern movement (arrows). **B:** The antennal responses to moving stripe patterns presented differently to the eyes. Pattern size and velocity as given in figure 2A. Twelve bees were tested in each group. **I.** Responses to a roll stimulus: one pattern is moving upward, the other one downward (drawing and arrows). **II.** The responses of the ipsilateral antenna are shown. **III, IV.** Responses to unilateral stimulation. The pattern moves either upward or downward (arrows) in front of only one eye. **III.** The contralateral responses (antenna of the non-stimulated side).

We also tested other directions of movement, but found no direction-specific antennal responses for lateral or longitudinal pattern movements below or above the bee.

Visual antennal responses can be elicited by patterns of different velocities and size (fig 3). The DAR is approximately the

same size for the velocities ranging between 25 °/s and 60 °/s (visual angle relative to the bee) (fig 3A). Stimulus patterns > 55° x 35° lead to maximum responses. Responses are relatively uniform when pattern velocities and sizes exceed their lower limits.

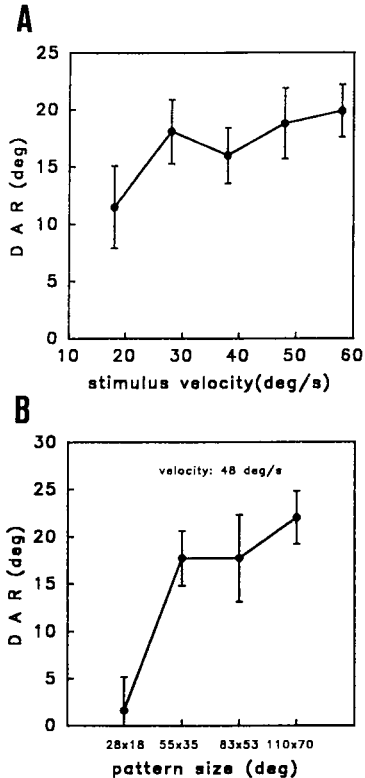


Fig 3. Velocity and pattern size dependence of visual antennal response (mean \pm SEM are shown). A: The direction-specific antennal response (DAR) to bilateral vertical stimulation with a moving stripe pattern (size 110° x 70°) at different velocities is shown on the ordinate; the abscissa indicates the stimulus velocity. Twelve bees were tested. B: The DAR for bilateral vertical stimulation with a moving stripe pattern of different sizes is shown on the ordinate; the abscissa indicates the pattern size relative to the bee's eye (size in direction of stimulus movement x size in direction perpendicular to stimulus movement). Nine bees were tested.

Olfactory antennal responses

Under laboratory conditions honeybees respond to olfactory stimuli with rapid scanning movements of the flagellum. Figure 4 shows the antennal response to a drop of water presented laterally to the bee. This response occurs even when the eyes are covered with paint. The behaviour did not occur when the syringe was presented without the drop of water. The ipsilateral antenna displays rapid scanning movements towards the source of stimulation. The contralateral antenna shows scanning movements of smaller amplitude. The antennal response to a drop of water is identical to that during stimulation with an odour. Apparently, the bee responds to the increased water vapour and often the proboscis is also extended during water vapour stimulation (fig 4). The water vapour stimulus can be presented at different loca-

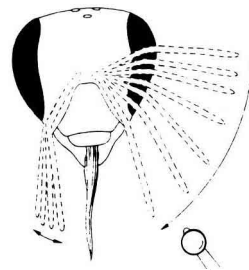


Fig 4. Drawing of a stroboscopic photo of a bee during stimulation with a drop of water. The flashes were delivered at a frequency of 12/s, exposure time: 500 ms; the water drop is indicated by the circle at the bottom. The bee also responded with proboscis extension to the stimulus.

tions relative to the antennae, thus stimulating either the right or the left antenna.

The spontaneous frequency of antennal passages across the 2 photodiodes before olfactory stimulation is $0.53 \text{ Hz} \pm 0.03$. A ventrally presented olfactory stimulus, in this experiment a scented air stream, elicits its rapid antennal scanning towards the source of stimulation. Figure 5A shows the relative responses of both antennae to different odorants which were all tested on the same group of bees. During stimulation, frequencies increase by a factor of between 1.23 (paraffin oil) and 1.95 (geraniol). The responses to isoamyl acetate, which forms part of the alarm pheromone of the bee, do not differ from the control stimulus in which paraffin oil was used as odorant. The responses to geraniol and caprylic acid differ significantly ($p < 0.05$, 2-sided Wilcoxon test), while the antennal response to citral shows an intermediate level. Antennal responses to different odorants apparently differ in their relative strengths.

This paradigm can be used for measuring the behavioural significance of different odorants in the laboratory and comparing them with olfactory responses of freely-moving bees (Pham-Delegue *et al.*, 1991). The behavioural thresholds for different odours can be calculated by measuring concentration-response relationships. Figure 5B shows this curve for caprylic acid, the odour which elicited the strongest response towards the stimulus source. The responses to the relative concentrations 10^{-2} and 10^0 differ significantly from the response to 10^{-4} ($p < 0.05$, 2-sided Wilcoxon test). Similar concentration-response relationships were found for citral and geraniol.

Mechanical antennal responses

Bees scan objects within the range of their antennae using brief and frequent contacts

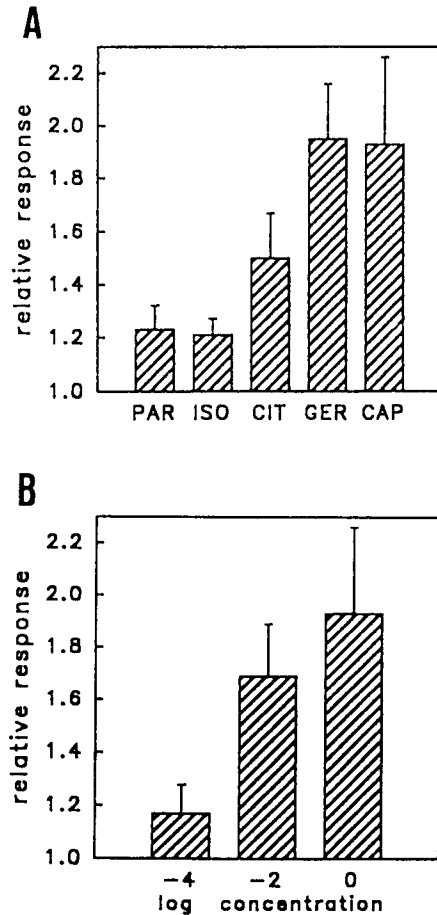


Fig 5. Quantification of the olfactory antennal reflex. The frequency of antennal passages was measured before and during olfactory stimulation. The ordinates show the mean relative response (antennal response frequency during stimulus/before stimulus) as measured by the device shown in figure 1B (\pm SEM). A: Relative responses to different odors. Fifty μ l of each pure odour was put on a filter paper. An airstream passing over the filter papers was presented to the bee as olfactory stimulus. The following odours were used: paraffin oil (PAR), isoamyl acetate (ISO), citral (CIT), geraniol (GER) or caprylic acid (CAP). Twenty bees were tested. B: Relative responses to different concentrations of caprylic acid. The abscissa shows the relative logarithmic concentration. Twenty bees were tested.

(fig 1C; Kloppenburg and Erber, 1989). Touching frequency varies considerably in different animals from $< 3/\text{min}$ to $> 300/\text{min}$. This response parameter appears to depend on the bees' state of arousal. After sensitisation of the bee with sugar water, the frequency of touching increases significantly ($p < 0.05$, Wilcoxon matched-pairs test) (Pribbenow *et al*, 1992).

Figure 6 shows the relative frequency distribution of touching duration in an experimental series of 10 bees. This distribution is typical of mechanical scanning behaviour. The most frequent touching durations are found in the range < 10 ms, whereas touching durations > 20 ms are relatively rare. In this experiment the mean value of the distribution was 7.5 ms and the median value 6 ms. In another series of experiments it has been shown that the touching duration does not differ significantly when the compound eyes are closed. Mechanoreceptors on the flagellum are not necessary for the control of the scanning movements. Bees can even

scan objects when the flagellum has been replaced by a small silver wire (Pribbenow *et al*, 1992).

Plasticity of the mechanical antennal response

The mechanical antennal response shows a high degree of plasticity. Preliminary experiments had shown that bees seem to learn the position of an object within the range of the antennae very fast. This observation was tested in a series of experiments with 10 bees. Plasticity of the response was found in most animals. Figure 7 shows a typical experiment with a single insect. The insect's eyes were blinded with black paint and a small object was placed close to the insect for 30 min. Antennal movements were recorded with a video camera before and after presentation of the object.

Without the presence of an object the antenna performs small and large scanning movements, most frequently in the fronto-ventral area (fig 7A). After the presentation of a square object the antenna scans the area where the object had been. The behaviour gives the impression that the antenna 'searches' for the object and scans the contours (fig 7B). Antennal scanning behaviour during the 2 min following the removal of the object differs significantly from the spontaneous antennal behaviour ($p < 0.0001$, χ^2 test). The experiments show that bee antennae scan objects by touching them briefly and frequently. This antennal behaviour shows plasticity.

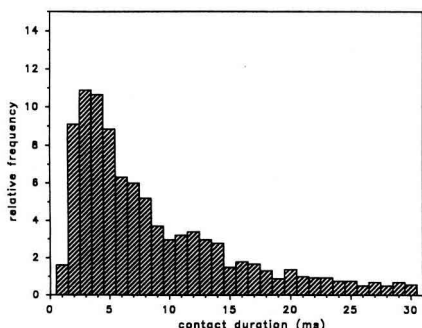


Fig 6. Frequency histogram of contact durations for touching a metal wire. The ordinate shows the relative frequency of contact durations the abscissa indicates the duration of the contacts. Ten bees were tested; 1 617 contacts were evaluated.

DISCUSSION

Honeybees respond with specific antennal movements to stimuli of different modalities. The antennal responses can be bilat-

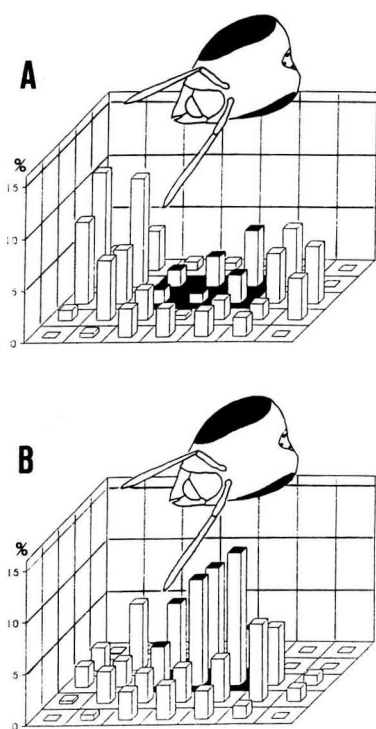


Fig 7. The plasticity of mechanical antennal scanning behaviour is shown, together with the relative position of the bee. Antennal movements were registered by a video camera which was placed lateral to the bee. Video recordings before (A) and after (B) presentation of an object positioned lateral to the bee were made and evaluated frame by frame. **A:** The relative frequency of the position of the antenna during scanning in the 2-dimensional plane is shown before an object was presented to the bee. The space being scanned by the antenna was divided into equal squares (1 x 1 mm). The ordinate shows the relative frequency of antennal positions for the different squares. Black areas indicate the position of the object presented later (object size: 3 x 2 mm). Bees most frequently scanned the fronto-ventral area. Measurements were made for 1 min. **B:** Antennal scanning after an object which had been presented to the bee for 30 min was removed. The first minute after removal is shown. The former position of the object is indicated in black. Bees preferentially scanned the area where the object was positioned.

eral, as during olfactory and gustatory stimulation, or unilateral, as during visual stimulation and when scanning an object (Erber and Schildberger, 1980; Erber, 1981; Kloppenburg *et al*, 1989). The motor programme of the antennae depends on the specificity and intensity of the stimulus (Ellerkmann *et al*, 1989). Since the same behavioural output can be used to study different sensory systems in the bee, antennal reflexes constitute a useful approach for neurobiological analyses. In addition, the behaviour is controlled by only a few muscles (6) and a small number of motoneurons (15) for each antenna (Kloppenburg, 1990).

If neurobiological experiments are to have behavioural significance, they should be performed under similar conditions to those of the behavioural analyses. Studies such as that presented here constitute very sensitive measures for the determination of appropriate stimuli or the identification of neuroactive compounds, their effective concentrations and sites of application (Erber *et al*, 1991).

The visual antennal reflex in the bee has been used to study neuromodulation in the optic ganglia. Serotonin reduces the directionally-specific antennal response both when the amine is applied over the entire brain or injected locally into the lobula. Octopamine application has the opposite effect. Both amines act over long periods (measured over 1 h) after application, indicating a neuromodulatory function of the 2 compounds. As the visual antennal response to a moving stripe pattern is specific for the stimulated side, the effects of amine injection into one hemisphere can be studied by stimulating the treated and the untreated side and evaluating the antennal responses separately (Kloppenburg, 1990; Erber *et al*, 1991).

Electrophysiological analyses of evoked potentials and recordings of single visual

neurons in the lobula have shown that the behavioural effects are due to the modulation of the directional specificity of movement-sensitive neurons (Kloppenburger, 1990). A straightforward electrophysiological analysis is possible by limiting the recorded neurons to direction-specific, vertical-movement sensitive cells and by using the neuroactive compounds, concentrations and injections sites which were effective in the behavioural experiment.

The problem of appropriate intensity and quality of the stimuli in the analyses of the olfactory system can be overcome by the experiments presented here (fig 5). The response profile for different odours, the effective concentration of the odorant and the appropriate delivery of the stimulus has been precisely defined in behavioural experiments.

The response to a drop of water is particularly useful for analyses of signal processing in the olfactory pathway of the brain. The response to increased vapour pressure is in all aspects identical to that during an olfactory stimulus. Treatments which affect olfactory responses have similar effects on the responses to a drop of water, indicating that signal processing for both stimuli is closely related (Erber, 1981; Mercer and Menzel, 1982; Menzel *et al*, 1988). As both antennae respond to a drop of water with coordinated movements directed towards the stimulus source, stimulation of one antenna can be compared to stimulation of the other antenna. Injection of neuroactive compounds into different parts of the mushroom bodies results in response changes on the treated side, while the antennal responses on the other side remain unaltered. Comparing the responses of the 2 antennae made it possible to show that the peptides FMRFamide and CCK have long-lasting effects in the α -lobe of the mushroom bodies (Erber *et al*, 1989; Kloppenburger *et al*, 1990a). This finding is important because neurons in

the mushroom bodies demonstrate FMRFamide-like and CCK-like immunoreactivity (Noble and Goodman, 1987; Schürmann and Erber, 1990). Further immunocytochemical analyses using antibodies against both peptides in the same preparation have demonstrated that immunoreactivity can be attributed to different subgroups of intrinsic mushroom body neurons (Kloppenburger *et al*, 1990a).

Behavioural experiments and neuroanatomical studies have shown that processing of olfactory information in the honeybee is side-specific (Martin, 1964; Masson, 1977; Erber *et al*, 1980). The physiological function of the tract connecting the antennal lobes (Masson, 1982; Arnold *et al*, 1987) has not been analysed so far. The experimental design for testing responses of both antennae after injecting small amounts of neuroactive agents into the olfactory pathway neuropile of one hemisphere has the advantage of constituting an intrinsic control. General behavioural effects that influence both hemispheres, such as arousal, result in response changes of both antennae. Specific effects due to the treatment of one hemisphere are detected by comparing the different responses of the 2 antennae.

Only recently has the response to a drop of water also been used to analyse the function of mechanoreceptors located at the antennal joints of the bee (Maronde, personal communication). Ablation of mechanosensitive hair plates at the antennal joints leads to uncoordinated movements of the 2 antennae during stimulation with a drop of water. This change in behaviour is a very sensitive measure for the function of mechanoreceptors on the antennae.

The mechanical antennal reflex is characterized by brief contacts of the antenna with an object. Neuroanatomical studies suggest that mechanosensory inputs from the antenna converge onto antennal moto-

neurons in the dorsal lobe (Kloppenburger, 1990). Electrical stimulation of the antennal nerve while recording action potentials in the nerve that innervates the antennal head muscles resulted in latency periods of 5 ms between the sensory stimulus and the response of the motoneurons. The brief contact duration of this reflex could therefore be the result of monosynaptic connections between mechanoreceptors and motoneurons. However, recent experiments have not been able to verify this hypothesis (Pribbenow *et al*, 1992). Antennal scanning of an object is most likely controlled by a motor programme initiating oscillatory movements of the antenna. Mechanosensory inputs are necessary to start this programme. It is then performed for some time without feedback from antennal mechanoreceptors.

The mechanical antennal reflex has recently been used to study neuromodulation in the antennal mechanosensory-motor neuropile of the dorsal lobe (Riens *et al*, 1992). Antennal contact durations can be measured and evaluated on-line by a computer. Contact durations are enlarged after local application of serotonin into the dorsal lobe. Octopamine injection, on the other hand, had no effect on the duration of antennal contact. The behavioural analysis of the mechanical antennal reflex is currently used for comparison of putative serotonin antagonists in the dorsal lobe.

The plasticity of the mechanical antennal reflex is a very interesting behavioural effect, not least because the mechanoreceptive inputs from the antenna, their projections to the motor neuropiles and the motoneurons involved are well characterized (Suzuki, 1975; Masson and Arnold, 1987; Kloppenburger, 1990). From earlier experiments it is known that antennal responses can be classically conditioned using either an odour or the movement of a stripe pattern as conditioned stimulus (Erber and Schildberger, 1980; Erber *et al*,

1984). An operant-conditioning paradigm has been described in which bees were rewarded with sugar water for maintaining different positions of the antennae (Erber *et al*, 1984). Thus, antennal reflexes appear to display a great amount of plasticity. It is therefore not surprising that the mechanical antennal response also shows a high degree of behavioural adaptivity. Though there are other antennal response behaviours showing plasticity, the "neural design" of the mechanical antennal reflex is simpler. A physiological analysis of the underlying mechanisms therefore appears feasible. The significance of the different types of mechanoreceptors on the antenna and the antennal joints in behavioural plasticity is currently being analysed in behavioural experiments.

The antennal responses described here certainly constitute only a small range of antennal responses which can be studied under laboratory conditions. Using these reflexes as tools for studying the nervous system seems to be of value for different sensory systems of the bee and also for studies on behavioural plasticity.

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Résumé — Les réflexes antennaires de l'abeille : un moyen d'étude du système nerveux. L'interprétation des analyses neuroéthologiques chez les arthropodes dépend des observations comportementales qui doivent être faites dans des conditions semblables à celles de l'expérimentation électrophysiologique. Chez l'abeille,

les réflexes du proboscis et de l'antenne remplissent cette condition. Les abeilles répondent par des réflexes antennaires aux stimuli de diverses modalités lorsqu'elles sont maintenues dans les conditions limitées d'une expérience de laboratoire. Les réponses antennaires aux diverses modalités de stimulation peuvent être quantifiées par un dispositif optoélectronique, par l'enregistrement électronique des contacts antennaires avec des objets et par des enregistrements vidéo (fig 1). Les insectes réagissent à des motifs rayés en mouvement vertical par des réponses antennaires directionnelles spécifiques. Des motifs rayés animés d'une vitesse de 25°/s et 60°/s déclenchent le maximum de réponses (fig 3A). La taille du motif minimal est de 55° x 35° par rapport à l'œil de l'abeille (fig 3B). Une stimulation unilatérale à l'aide du motif provoque des réponses antennaires ipsilatérales (fig 2). Les abeilles répondent à des stimuli olfactifs par des mouvements de balayage dirigé des 2 antennes. L'ampleur de la réponse dépend du logarithme de la concentration de l'odeur. Les réponses varient selon les classes de produits odorants (fig 5). Les abeilles touchent rapidement et fréquemment des objets avec leurs antennes. La durée moyenne du contact est inférieure à 10 ms (fig 6), et la fréquence des contacts va de moins de 3/min à plus de 300/min. La réponse antenneuse mécanique présente un degré élevé de plasticité. Les abeilles semblent apprendre la position d'un objet, et balaient la région et les contours de l'objet pendant au moins 2 min après qu'il a été retiré (fig 7). Les réflexes antennaires de l'abeille servent d'outils pour étudier le traitement du signal, la neuromodulation, la sensibilisation et l'apprentissage dans divers systèmes sensoriels.

***Apis mellifera* L / réflexe antenneuse / plasticité / neurobiologie**

Zusammenfassung — Antennenreflexe bei der Honigbiene: Mittel zum Studium des Nervensystems. Die Interpretation neuroethologischer Experimente ist davon abhängig, daß die Verhaltensbeobachtungen unter den gleichen Bedingungen wie die elektrophysiologischen Analysen durchgeführt werden. Wenn Honigbienen unter eingeschränkten Laborbedingungen gehalten werden, antworten sie mit Antennenreflexen auf Reize unterschiedlicher Modalität. Rüssel- und Antennenreflexe der Biene erfüllen diese Voraussetzungen. Die Antennenreaktionen auf Reize unterschiedlicher Modalitäten können quantitativ mit einer optoelektronischen Meßeinrichtung, durch elektronische Registrierung antennaler Kontakte mit Gegenständen und durch Videoaufnahmen ausgewertet werden (Abb 1). Die Tiere reagieren mit richtungsspezifischen Antennenreaktionen, wenn ihnen ein vertikal bewegtes Streifenmuster geboten wird. Maximale Reaktionen werden von Streifenmuster mit Geschwindigkeiten zwischen 25°/s und 60°/s ausgelöst (Abb 3A). Die minimale Mustergröße beträgt 55° x 35° relativ zum Komplexauge der Biene (Abb 3B). Einseitige Reizung führt zu ipsilateralen Antennenreaktionen (Abb 2). Bienen reagieren auf olfaktorische Reize mit gerichteten Bewegungen beider Antennen. Die Antwortstärke ist vom Logarithmus der Duftkonzentration abhängig. Verschiedene Duftklassen führen zu abgestuften Reaktionen (Abb 5). Bienen berühren Objekte mit ihren Antennen kurz und mit hoher Frequenz. Die mediane Berührungsdauer ist < 10 ms (Abb 6), die Berührungshäufigkeit schwankt zwischen < 3/min bis > 300/min. Die mechanische Antennenreaktion zeigt einen hohen Grad an Plastizität. Bienen können die Position eines Objektes lernen, sie tasten die Fläche und Konturen des Objektes etwa noch 2 min, nachdem das Objekt entfernt worden ist, ab (Abb 7). Die Antennenreflexe der Bienen sind wertvolle Hilfsmittel, um

die Signalverarbeitung, Neuromodulation, Sensitivierung und Lernen in verschiedenen sensorischen Systemen zu untersuchen.

Antennenreflex / Plastizität / Neurobiologie / *Apis mellifera*

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