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INTRACELLULAR RECORDINGS OF NECK MUSCLE MOTONEURONES DURING EYE CLEANING BEHAVIOUR OF THE CRICKET

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SUMMARY

Intracellular recordings were made from prothoracic neurones of crickets which were free to move in a quite normal way. During the head roll component of eye cleaning, the motoneurones (MNs) to the driving muscles, dorso-ventral neck muscles 55, 56 and 60, received excitatory and/or inhibitory input from several spiking neurones. The discharge patterns of these MNs were basically identical to those in intact animals.

Lesion of connectives showed that the motor pattern of eye cleaning is generated by a complex neural network in the prothoracic and the suboesophageal ganglia, in which both neck connectives are involved.

Single electric shocks to the axons of the interommatidial mechanoreceptors through which eye cleaning is elicited evoked an EPSP followed by an IPSP with a latency of about 10 ms in all MNs of the three muscles on both sides. During repeated stimulation of the receptors this input is predominantly inhibitory.

INTRODUCTION

The description of connections between identified neurones, using combined electrophysiological and neuroanatomical techniques, has clearly helped our understanding of the neural basis of behaviour (e.g. Robertson & Pearson, 1984; Reichert & Rowell, 1985; Krasne & Wine, 1984; Burrows, 1981; Davis & Kovac, 1981). However, in most of the studies the animals were extensively dissected and/or restricted in their mobility, and often only 'virtual behaviour' was possible, i.e. coordinated motoneurone (MN) activity after denervation. In this paper a preparation is introduced which allows intracellular recordings from prothoracic neurones of crickets while the animals are free to move and to perform many different behaviour patterns in a relatively normal way. This preparation was used to investigate the neuronal control of the head roll component of eye cleaning behaviour of the cricket *Gryllus campestris*.

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Key words: behaving cricket, eye cleaning behaviour, intracellular recordings, motoneurones.

Eye cleaning in crickets is a well defined behaviour pattern (Honegger, Reif & Müller, 1979; Hensler & Honegger, 1985). It can be elicited by a specific input, i.e. mechanical stimulation of interommatidial bristles on the compound eye and consists of the above mentioned head roll towards the stimulated eye, followed by a cleaning movement performed by the ipsilateral foreleg (Honegger *et al.* 1979). The interommatidial mechanoreceptors which receive the stimulus project to the ventral neuropile of the suboesophageal and prothoracic ganglion (Honegger, 1977), the ganglia which also contain the bulk of neck muscle MNs (Honegger *et al.* 1984).

As a first step in the analysis, Hensler & Honegger (1985) measured the discharge patterns in the motor nerves of six prominent dorso-ventral and dorsal-longitudinal neck muscles during eye cleaning in intact animals. In this paper, the discharge patterns during eye cleaning, as measured intracellularly in dissected crickets, are presented for the MNs of the dorso-ventral neck muscles (M) 55, M56 and M60, which are shown to be the main contributors to head roll movements. From experiments in which connectives were severed and from the nature of the subthreshold activity in the MNs, information was gained about the organization of the neural network underlying eye cleaning. The properties of some interneurones of the network will be described in a following paper (K. Hensler, in preparation). Furthermore, it is shown that in addition to eye cleaning behaviour, stimulation of the interommatidial mechanoreceptors elicits an inhibitory input of short latency to all the neck muscle MNs investigated. Some characteristics of this pathway are described.

MATERIALS AND METHODS

All experiments were carried out with adult *Gryllus campestris* of either sex, either from our laboratory culture or from the field.

Preparation

A special holder was developed for recording intracellularly from neurones of the prothoracic ganglion in behaving animals (Fig. 1A). The U-shaped middle part of the holder was placed between the pro- and mesothorax. From each side a moveable piston was waxed onto the side wall of the pronotum (hatched area in Fig. 1A). The anterior third of the pronotum was slit open along the dorsal midline, and from the posterior two-thirds a piece of cuticle, about 2 mm wide, was removed. The two halves of the pronotum were drawn apart with the pistons (1–2 mm) which were then locked with screws.

All tissue overlying the prothoracic ganglion was removed, including the longitudinal neck muscles 50/51, 52 1/2, 53 1/2 and 65 (numbering system after Honegger *et al.* 1984), the ventral parts of the pleural apophysis with the muscles attached to it, and the gut, the anterior part of which was ligatured. Great care was taken not to damage the four main tracheae.

The most critical step was to manipulate the ganglion on to a silver spoon with grooves for the connectives and the side nerves. When side nerves or connectives

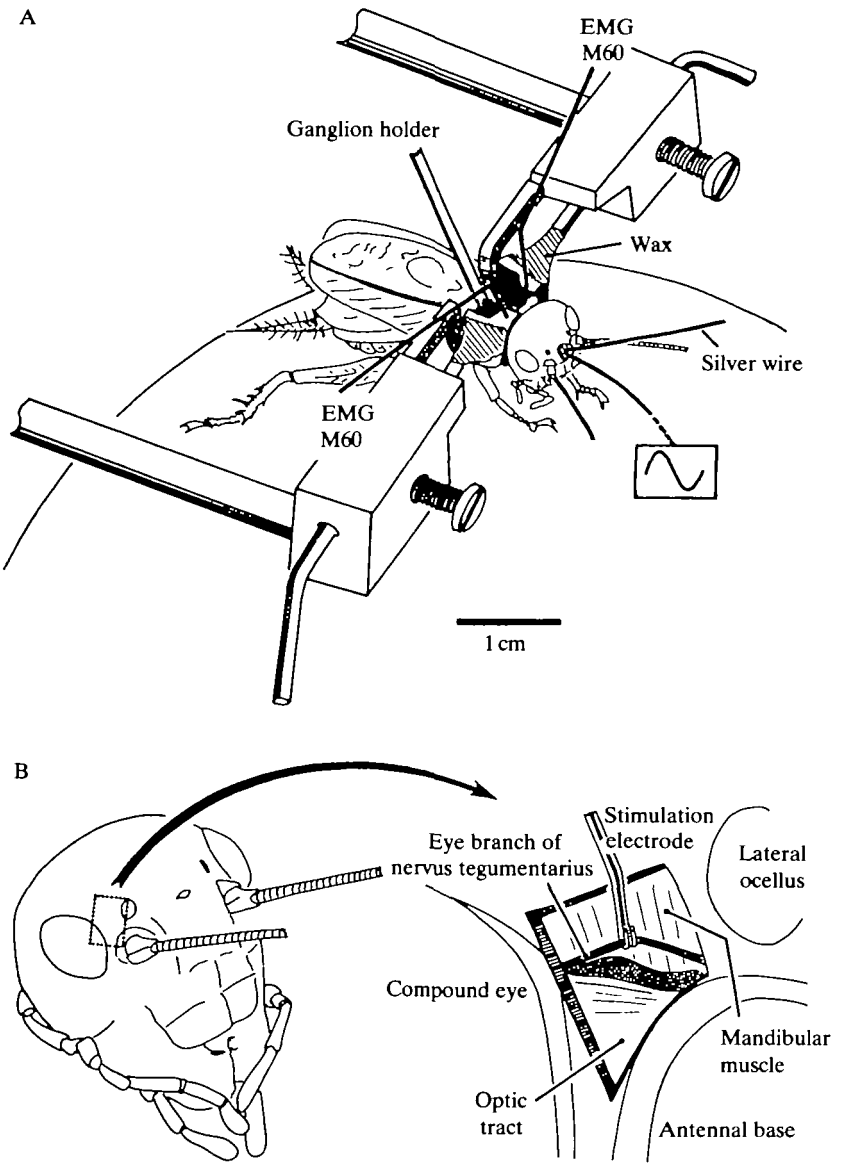


Fig. 1. (A) Schematic diagram of the preparation used for intracellular recordings from prothoracic neurones in behaving crickets. The animals were mounted in a special holder over a walking-sphere. Hatched area: region where the pistons of the holder were waxed onto the side walls of the pronotum. EMG M60: electromyogram electrodes at the insertion points of muscles 60. The silver wire is part of a device for measuring head roll movements. At the dotted end it is connected to a frequency generator. (B) Position of the double hook electrode used for stimulating the nerve which contains the axons of the interommatidial mechanoreceptors. For preparation procedure see Materials and Methods.

were stretched too much the animals became inactive and unresponsive to stimuli. Finally the ganglion was glued on to the spoon, with small drops of tissue glue

(histoacryl blue, Braun). This procedure prevented all movements of the ganglion and allowed stable recordings even in vigorously struggling animals. When the ganglion was fixed in such a way it was possible to cut neck connectives without losing a penetrated neurone.

Normally the ganglion sheath was softened with collagenase (Sigma Type VIII, high purity), a few crystals being applied in a drop of saline for 10–20 min. During the experiments the crickets could walk on a styrofoam ball floating on an air cushion (Dahmen, 1980) and except for the fixed pronotum were free to move all parts of their body including the head.

Stimulation of interommatidial mechanoreceptors

Eye cleaning behaviour was usually elicited by brushing over the eye with an eyelash glued onto a holder, thereby stimulating the interommatidial mechanoreceptors. In some preparations more defined stimuli were applied electrically to the axons of these receptors, which leave the optic lobe as a separate nerve and enter the brain *via* the dorsal tegumentary nerve (Honegger, 1977; cf. Fig. 9A). Onto this nerve a double hook electrode of varnish-insulated 30-μm steel wire was placed through a small window cut into the head capsule (Fig. 1B). After the electrode had been installed, the removed piece of cuticle was reinserted and the wires were waxed to the dorsal, posterior mid-part of the head. The wires were mounted in a wide loop to the holder on the pronotum, so that they did not affect the mobility of the head. Stimuli of 1–5 V with a duration of 0·3 ms were applied *via* a stimulus isolation unit.

Recordings

Intracellular recordings were taken from the main neuropilar processes of neck muscle motoneurones using glass micropipettes with a resistance of 50–100 MΩ when filled with 5 % Lucifer Yellow (Stewart, 1978) in distilled water in the tip and with 0·2 mol l^{-1} lithium chloride in the shaft. Electromyograms (EMG) from both muscles 60 were recorded with steel needles from their insertion points at the pronotum (Fig. 1A). The silver spoon supporting the ganglion served as the reference electrode. During the experiment the ganglion was bathed in a mixture of haemolymph and saline (after Eibl, 1978; without glucose).

For measuring head rolls a silver wire was waxed obliquely forward onto the head (Fig. 1A). Its position between two lateral electrodes (not shown in Fig. 1A) was measured using the method of Sandeman (1968). For more details see Hensler & Honegger (1985).

Evaluation of data

Signals were amplified and displayed conventionally and stored on magnetic tape. Later, hard copies of relevant sequences were obtained *via* a chart recorder. In some stimulation experiments postsynaptic potentials were averaged using a digital oscilloscope (Vuko VKS 22-16) connected to an Apple IIe desk computer.

Head rolls following mechanical eye stimulation were accepted as part of eye cleaning behaviour with basically normal motor output when, first, all movement components of eye cleaning were observed and, second, the motor pattern of both muscles 60 as measured by myograms was similar to that in intact animals (Hensler & Honegger, 1985).

Staining methods

Lucifer Yellow was injected into the motoneurones with constant hyperpolarizing current of 5–10 nA for about 5 min. The ganglion was dissected out and fixed in 4% formaldehyde in 0·2 mol l^{-1} phosphate buffer (pH 7·2) for 30 min, dehydrated in ethanol and cleared in methylsalicylate. The stained neurones were viewed as whole mounts and photographed in different planes of focus with a Leitz fluorescence microscope. Drawings were made from projected slides.

RESULTS

Identification and properties of motoneurones

The MNs (motoneurones) of M (muscle) 55, M56 and M60 lie in the prothoracic ganglion (PTG) (Honegger *et al.* 1984). Their main neurites were penetrated 100–200 μm below the dorsal surface of the PTG, in the region marked by a stippled circle in Fig. 2. MNs were easily distinguishable from other neurones of this region by the steady barrage of postsynaptic potentials (PSPs) which they received in quiescent animals (Fig. 3, upper trace). The PSPs often occurred in groups and most of them were inhibitory (IPSPs) as shown by injecting hyperpolarizing current through the recording electrode: after weak hyperpolarization most of the PSPs disappeared (Fig. 3, middle trace) and after stronger hyperpolarization they re-appeared with a reversed sign (Fig. 3, lower trace) (cf. Pearson, Wong & Fourtner, 1976). In quiescent animals, none of the MNs was found to discharge tonically.

Motoneurones of muscles 55 and 56

M55 and M56 (inset Fig. 4) are innervated by three and four MNs respectively (common inhibitory neurones are not counted), which are similar in shape but differ in their fine structure (Honegger *et al.* 1984). One typical example is shown in Fig. 2 (left). When stimulated artificially by passing depolarizing current through the recording electrode, single MNs of both M55 and M56 caused a head roll movement to their ipsilateral side (Fig. 4A,B). Both muscles appeared to have very similar effects. The muscle to which an MN belonged had to be determined by comparing its morphology with the morphology described by Honegger *et al.* (1984).

The amplitude of the head roll depended on the discharge frequency and on the type of the MN: single spikes in fast type MNs (e.g. Fig. 4A) caused a short, small head twitch, and high frequency discharge (150 Hz or more) of these MNs could cause a head roll of up to 50°. Single spikes of slow type MNs had no visible effect

and high frequency activity caused a head roll of only 10°–30°. Most of the penetrated MNs of the two muscles were of a type between these two extremes (e.g. Fig. 4B).

In all cases the head was rolled relatively fast during the first 200–300 ms of depolarization. During this time 80–90% of the respective final roll angle was accomplished. Full deflection was then slowly approximated within several seconds (Fig. 4A,B). When the depolarization was switched off, the head either returned to the normal position (Fig. 4A) or a certain head roll (10°–20°) was maintained (Fig. 4B). In such cases the head was actively reset to the normal position (note the

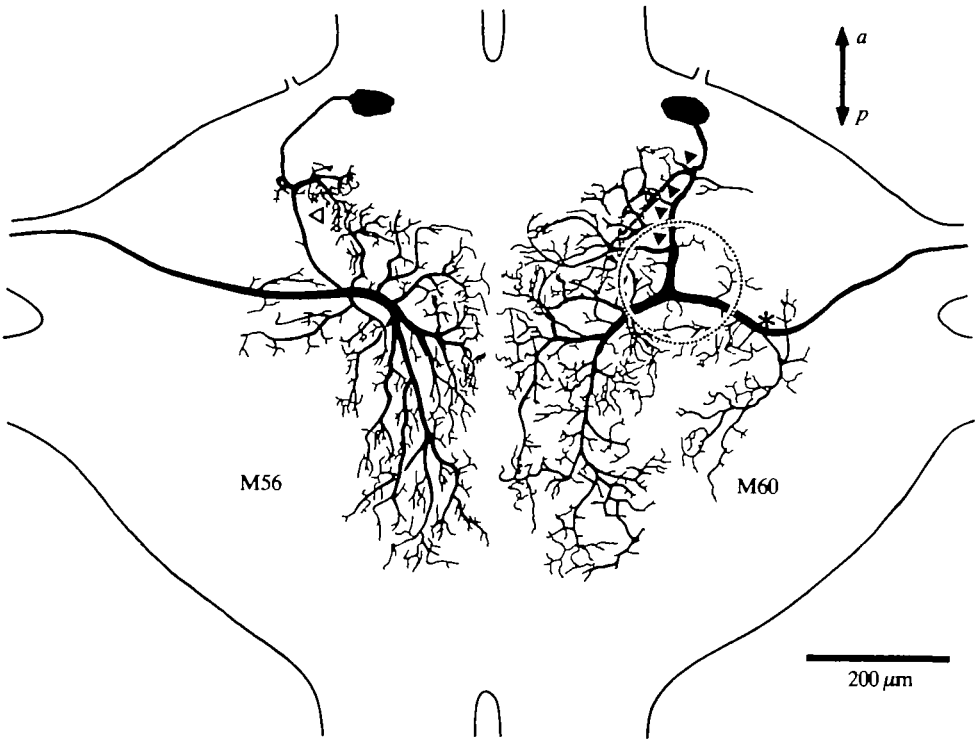


Fig. 2. Morphology of neck muscle motoneurones (MN) in the prothoracic ganglion (dorsal view), drawn after Lucifer Yellow fills. Left side: MN of muscle 56 as an example of the seven MNs of muscles 55 and 56 which are similar in shape. Right side: single excitatory MN of muscle 60. The main arborizations of both cells are in the dorsal neuropile regions. The somata of both cells lie in the middle horizontal plane and the axons of both cells leave the ganglion through nerve 3. The small branching field which originates from the anterior neurite of the MN of muscle 56 (open triangle) lies more ventrally than the main branching field. From the anterior neurite of the MN of muscle 60 several branches (closed triangles) originate in different planes of the ganglion, those originating closer to the soma lie more ventrally than those originating distal to the soma. Typical features of this MN are the bend at the position where the axon extends to the main neurite (asterisk) and the lateral posterior branch originating there (for details of the MN anatomy see Honegger *et al.* 1984). The dotted circle marks the region from which intracellular recordings were taken.

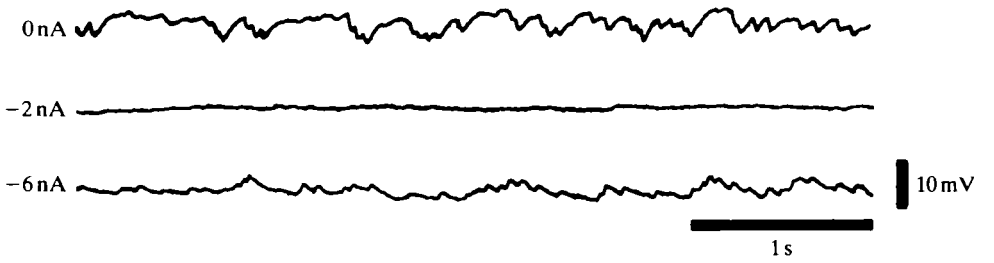


Fig. 3. Typical pattern of postsynaptic potentials (PSPs) received by the neck muscle motoneurones of a quiescent cricket (upper trace). Injection of hyperpolarizing current through the recording electrode revealed that the PSPs were inhibitory: with increasing current strength they first disappeared (middle trace) and then reappeared but with a reverse sign (lower trace).

two single spikes in Fig. 4B) before the animal moved or after applying an external stimulus like an air puff (arrow in Fig. 4B).

The physiological properties of the MNs of M55 and M56 (fast, slow) could not be correlated with the morphological types found by Honegger *et al.* (1984). The fine structure of an MN varies between individual animals, and single stained MNs, as obtained in the experiments described here, are difficult to classify without the other MNs of the respective muscle as a reference.

Motoneurone of muscle 60

M60 (inset Fig. 4) has only one excitatory MN, which is easily identifiable by its structure (Fig. 2, right). During the recordings this MN was identified by electromyograms (EMG) taken from the M60s on both sides (Fig. 1A). Spikes in the MN were followed one-to-one in the EMG (Figs 5–8) with a latency of 2–3 ms. Artificial excitation of the MN by injecting depolarizing current caused a head roll as well, but to the side contralateral to the excited muscle (Fig. 4C). The roll angle depended on the discharge frequency and could reach a maximum value of about 20° when the frequency reached 150 Hz. The course of the movement was similar to that for M55 or M56.

In summary, the MNs of M55 and M56 on one side of the ganglion and the MN of M60 on the other side of the ganglion can act together in producing a head roll to the side ipsilateral to M55 and M56.

Motoneurone activity during eye cleaning behaviour

In dissected animals, mechanical stimulation of the eye could elicit eye cleaning behaviour which appeared normal. Often the movements were not as marked and as precise as in intact animals (Hensler & Honegger, 1985), but the basic movement pattern was unequivocally the same. The latency from the start of the stimulation to the start of the behaviour was variable but normally longer than half a second (Fig. 7, black bars).

Motoneurone of M60

In intact animals the single excitatory MN of M60 displayed the most striking discharge pattern of all the MNs recorded from (Hensler & Honegger, 1985). Ipsilateral to the side of eye cleaning it released only a few spikes during return movements of the deflected head, but contralaterally it was activated with the highest mean frequency of all MNs recorded from, modulated with the rhythm of head

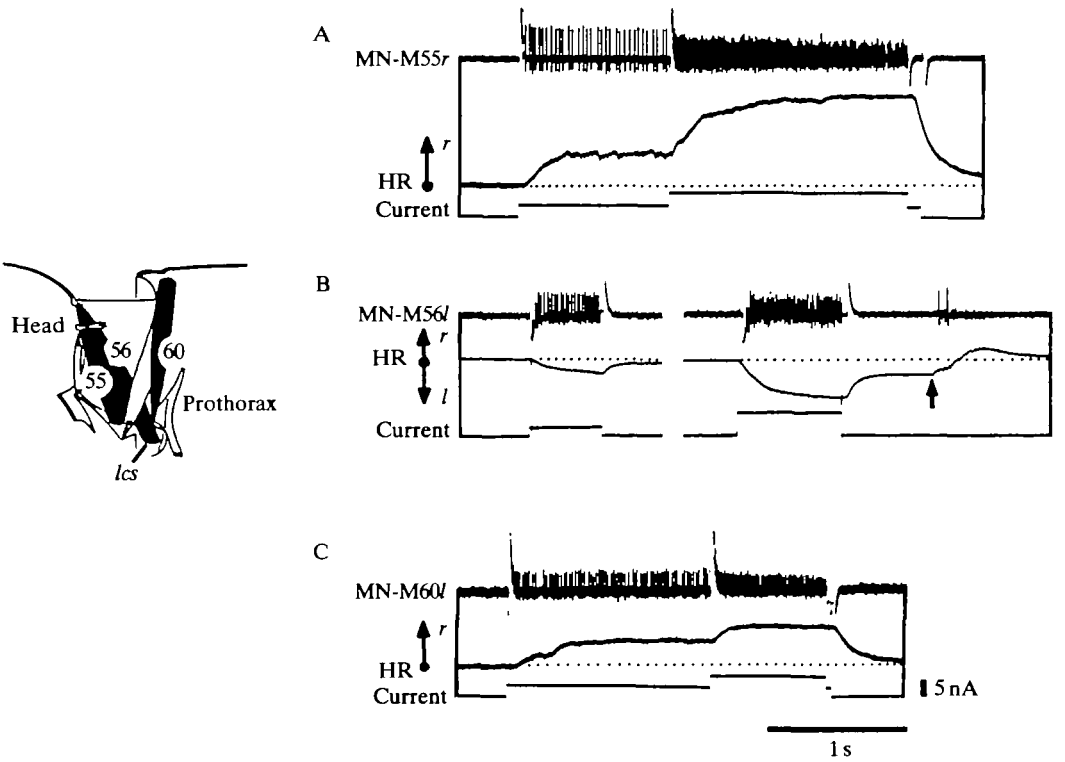


Fig. 4. Effect of activity in single motoneurones (MNs) of the dorsoventral neck muscles 55 (A), 56 (B) and 60 (C) on the head position. The MNs were stimulated artificially by injection of depolarizing current of variable strength (lower traces). The inset shows the position of the three muscles (drawn black) in the neck region (sagittal section, inner view, after Honegger *et al.* 1984). All three muscles insert on the lateral cervical sclerite (*lcs*). M55 and M56 run to the posterior region of the occipital ridge; M60 runs to the anterior, dorsal median rim of the pronotum. Upper traces: intracellular recording from the MNs *via* a high pass filter. Middle traces: measure of head roll movements (HR) from the normal position (closed circle and dotted line) to the right (*r*) or to the left (*l*). (A) Activity in an MN of the right M55 caused a head roll to the right. (B) Activity in an MN of the left M56 caused a head roll to the left. In both cases the strength of the head roll depended on the discharge frequency. When the MNs were switched off, in A the head returned to the normal position, in B the head returned incompletely and maintained a certain roll angle. A short wind puff onto the body (arrow) caused an active reset (note the two spikes in the MN) to the normal position. (C) When activated the single excitatory MN of the left M60 caused a slight, frequency-dependent head roll to the right, i.e. to its contralateral side. The recordings from A and C are from successive penetrations in the same animal. The strength of their head roll is directly comparable.

oscillations which were concurrent with the cleaning strokes of the ipsilateral foreleg (waves of about 4 Hz in the movement recordings of Figs 5–8). This discharge pattern appeared to be specific for eye cleaning behaviour and therefore can serve as an indicator for the activity of the eye cleaning motor programme.

In the dissected animals basically the same discharge pattern was found (Figs 5A,B,C, 8A; myograms in Figs 6A,B, 7A,B). However, the mean discharge frequency as compared with intact animals was reduced: ipsilaterally from 12·2 ± 7 Hz to 2·15 ± 2·8 Hz (20 cleaning sequences in five animals) and contralaterally from 138 ± 27 Hz to 96·7 ± 22 Hz (43 cleaning sequences in eight animals, measured for the first 200 ms of head roll). These values were obtained from myograms of M60s whose MNs had not been penetrated (penetration may change spiking activity of neurones due to injuries by the electrode).

During eye cleaning ipsilateral to the recording site, the MN of M60 was inhibited throughout the deflection phases of the head (asterisks in Fig. 5A,B), while the contralateral M60 (EMG) was excited. The inhibition is clearly demonstrated in Fig. 5B, where the artificially induced discharge of the MN (injection of depolarizing current) was reduced or disappeared during head deflections. Higher magnification of the recordings showed high frequency bursts of small, discrete IPSPs. Sometimes the inhibition appeared to suppress excitatory input (Fig. 8A).

During eye cleaning contralateral to the recording site, the MN received strong depolarizing input which elicited bursts of action potentials in correlation with the deflection phases of the head (Fig. 5C). The depolarizations were composed of many discrete EPSPs, which probably originated from different sources, as different EPSP amplitudes were found. During the legstroke-coupled rhythmic return movements of the head the MN was repolarized with the participation of IPSPs, as indicated by sudden potential drops (arrowheads Fig. 5C).

Motoneurones of muscles 55 and 56

In intact animals at least six MNs of M55 and M56 were activated during eye cleaning ipsilateral to the recording site, and 1–3 MNs were activated, but not as strongly, during eye cleaning on the contralateral side (Hensler & Honegger, 1985). It is not known whether all the morphologically identified cells (Honegger *et al.* 1984) were contained in the 42 MNs of M55 and M56 which were penetrated, but it seems to be probable as different types could be distinguished by the strength of the head roll they were able to produce (see previous section), and all variations of discharge patterns known from the intact animals were found.

During eye cleaning ipsilateral to the recording site, all MNs of M55 and M56 were excited, but with different strength (examples in Figs 6A, 7A). They received depolarizing input composed of different EPSPs which often was correlated with the deflection phases of the head. As in the MN of M60, the EPSPs seemed to originate from different sources as different amplitudes were found. During the return movements of the head the MNs were often repolarized with the participation of IPSPs as indicated by sudden potential drops (arrowheads in Figs 6A, 7A).

During eye cleaning contralateral to the recording site, the MNs behaved differently. One group was active, but weaker when compared with ipsilateral eye cleaning (Fig. 6B). A second group was inactive or even inhibited as shown by the reduction of artificially induced spiking (asterisks in Fig. 7B).

The activity patterns of all MNs of M55 and M56 recorded from were similar to the two examples shown here. The main differences between different MNs were restricted to various strength of activation.

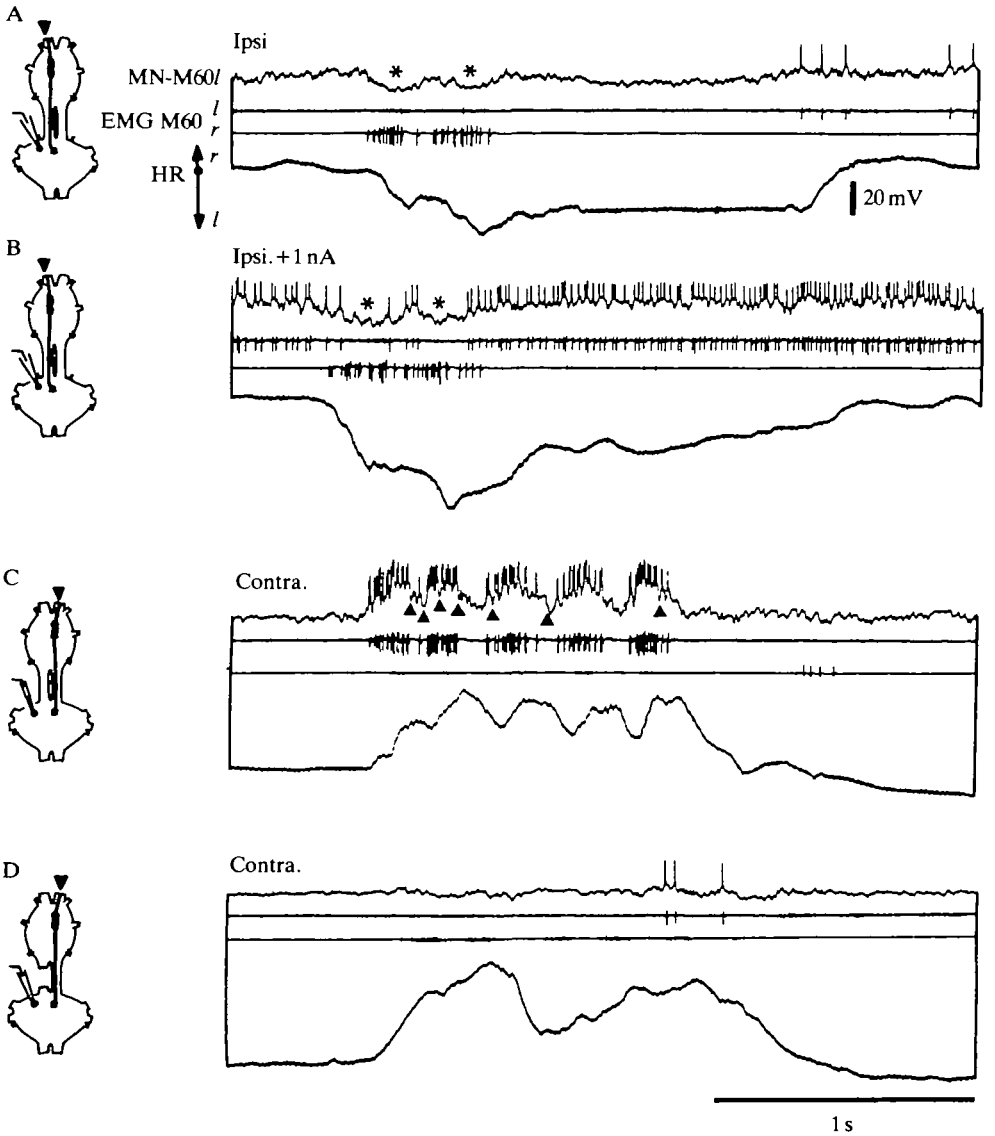


Fig. 5

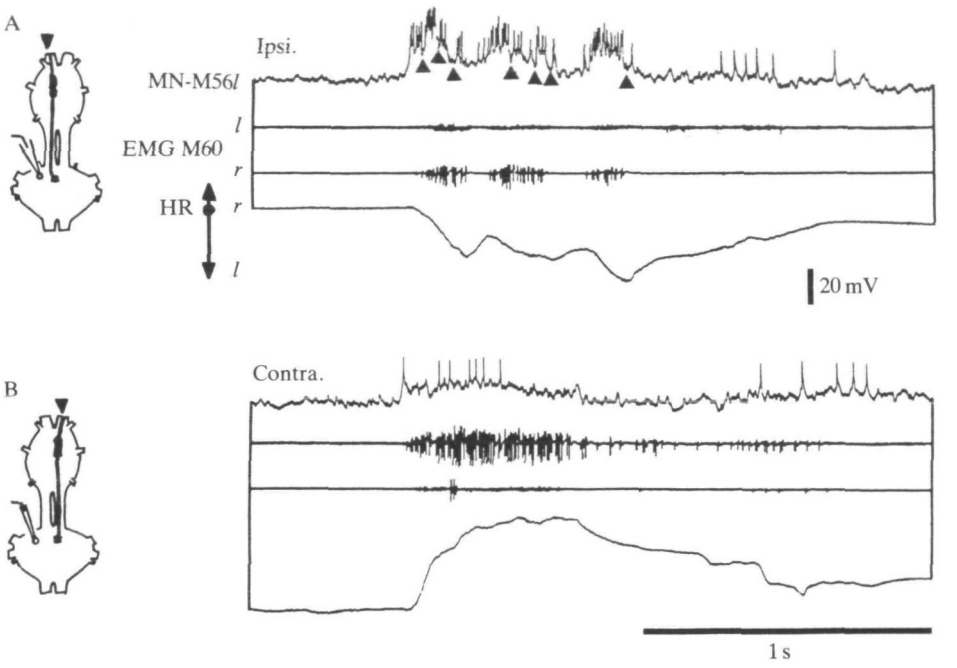


Fig. 6. (A) Intracellular activity in a motoneurone (MN) of the left M56 during eye cleaning, ipsilateral (Ipsi.) to the recording site. The MN was strongly activated by volleys of EPSPs with different amplitudes. Sometimes IPSPs occurred (arrowheads). (B) Eye cleaning contralateral (Contra.) to the recording site. The MN received EPSP-input but weaker than in A. The EMGs of both M60s serve as indicator for a normal motor programme of eye cleaning. Abbreviations, traces and insets as in Fig. 5.

Fig. 5. Intracellular activity in the single excitatory motoneurone (MN) of the left M60 during eye cleaning behaviour. Insets show the suboesophageal and the prothoracic ganglion with the side of stimulation (i.e. the direction of head roll, arrowhead), the course of the sensory axons through which eye cleaning is elicited (cf. Fig. 9A), the recording site and in D the position where a neck connective was severed. Eye cleaning started with a head roll (HR) from the normal position (level at the closed circle between the arrows) to the left (*l*) or to the right (*r*). Upper traces: intracellular recordings from the main neurite of the MN; middle traces: electromyograms (EMG) from the right (*r*) and the left (*l*) M60. (A) Eye cleaning ipsilateral (Ipsi.) to the MN recorded from. During head deflection phases the MN was inhibited (asterisks). (B) Same as A but the MN was pre-excited artificially by passing depolarizing current through the recording electrode to demonstrate the inhibition (asterisks) more clearly. (C) Eye cleaning contralateral (Contra.) to the MN recorded from. The MN was strongly excited by volleys of EPSPs with different amplitudes. The strength of the depolarizations was modulated with the rhythm of head oscillations, in correlation with repeated leg strokes over the stimulated eye. IPSPs (arrowheads) occurred shortly before and during return movements of the head. (D) Same as C but the neck connective on the side of the penetrated MN was cut (see inset). During eye cleaning on the intact side the strong depolarization of the MN as seen in C was abolished. Only few postsynaptic potentials and some spikes shortly before the final return movement of the head are visible.

Routes of information transfer to the motoneurones

Head rolls during eye cleaning involve neck muscle MNs in both halves of the PTG and the SOG (suboesophageal ganglion) (previous section and Hensler & Honegger, 1985). Thus, the two ganglia must be synchronized and information must be transferred to the side contralateral to the input from the interommatidial sensilla (projections see Fig. 9A) through which the behaviour is elicited. Some pathways of information transfer were elucidated by severing connectives. For these experiments the activity of an MN during eye cleaning was recorded while both neck connectives were intact. Then one of the connectives was cut, without removing the electrode from the MN, and the measurement was repeated. On the side where the connective

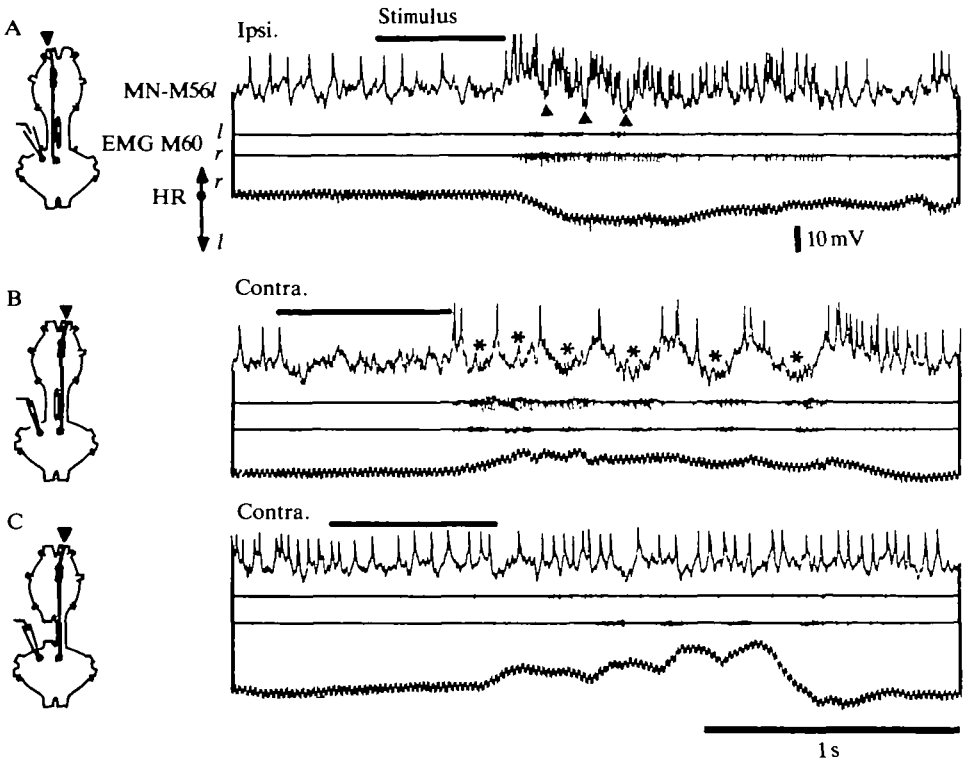


Fig. 7. Intracellular activity in a motoneurone (MN) of the left M56 during eye cleaning. The MN was pre-excited by +0·5 nA of injected current to visualize inhibition (cf. Fig. 5B). Black bars indicate approximately the time of mechanical eye stimulation with an eyelash. (A) Eye cleaning ipsilateral (Ipsi.) to the recording site. The MN was inhibited during the stimulation and strongly excited during the head roll. (B) Eye cleaning contralateral (Contra.) to the recording site. The MN was inhibited during the stimulation. During the head roll it was also inhibited, phase coupled to head oscillations. (C) The same experiment as in B but the left neck connective was cut (see inset). The inhibition as seen in B was abolished. Note the discharge patterns of M60 (EMG). EPSP amplitudes were decreased by the injected current. Abbreviations, traces and insets as in Fig. 5.

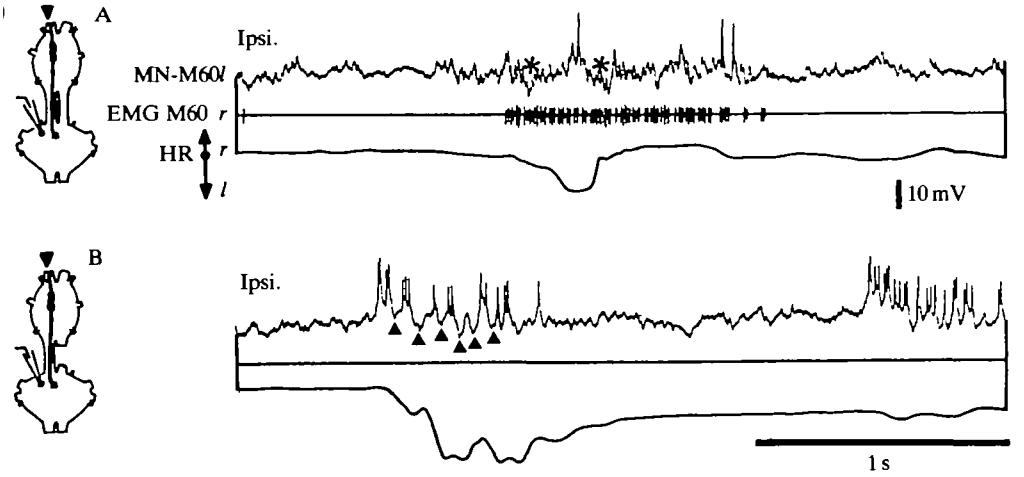


Fig. 8. Intracellular activity in the motoneurone (MN) of the left M60 during eye cleaning ipsilateral to the MN, A before and B after the neck connective contralateral to the penetrated MN was severed (see inset). (A) Volleys of IPSPs (asterisks) occurred mixed with excitatory input. (B) Only few IPSPs (arrowheads) were left, excitation was dominant. The excitation in the contralateral M60 (EMG) was abolished. Abbreviations and inset as in Fig. 5.

was severed eye cleaning could no longer be elicited, but on the intact side apparently normal eye cleaning was possible (cf. Hensler & Honegger, 1985).

Motoneurone of muscle 60

When the neck connective was cut on the side where the MN of M60 was impaled (inset Fig. 5D), the normally occurring strong excitation of this MN during eye cleaning on the intact side (Fig. 5C) was abolished. Only weak PSP input remained (Fig. 5D, see also EMG recording in Figs 7C, 8B). When in similar experiments the neck connective contralateral to the impaled MN was cut and eye cleaning was elicited on the intact side (inset Fig. 8B), i.e. ipsilateral to the recorded MN, most of the inhibition of the MN which was found in intact animals (Figs 5A,B, 8A) disappeared and an excitation became apparent (Fig. 8B).

These experiments show that during eye cleaning an essential part of the excitation of the MN of M60 contralateral to the side of eye cleaning, and an important part of the inhibition of the MN of M60 ipsilateral to the side of eye cleaning, must depend on the activity of intersegmental interneurones which project through the neck connective contralateral to the side of eye cleaning, i.e. contralateral to the side where the sensory information from the interommatidial sensilla descends to the PTG (cf. inset Fig. 8B).

Motoneurones of muscles 55 and 56

When one neck connective was cut, neither strength nor general shape of the head roll was changed during eye cleaning on the intact side, compared with the head roll

before the lesion (Figs 5D, 7C, 8B). Thus, it can be concluded that during eye cleaning the input to the MNs of M55 and M56 on the intact side, i.e. ipsilateral to the head roll, was not essentially influenced by the lesion. However, the main part of the inhibition, found in some MNs of intact animals on the side contralateral to the head roll (Fig. 7B), was usually abolished (Fig. 7C) by the lesion (inset Fig. 7C). In principle the same was found for many of those MNs of M55 and M56 on this side which normally received excitatory input during eye cleaning (e.g. Fig. 6B). Hence, at least part of the input to the MNs of M55 and M56 contralateral to the side of eye cleaning must depend on intersegmental interneurones in the neck connective on this side.

Furthermore it was tested whether the MNs themselves could influence each other, as is known for several MNs in the locust (Hoyle & Burrows, 1973; Kendig, 1968) and the cricket (Bentley, 1969). Antidromic stimulation of the MNs of M55, M56 and M60 never evoked PSPs in MNs of the contralateral hemiganglion, and antidromic stimulation of MNs of M55 and M56 never evoked PSPs in the MN of M60 and *vice versa*. However, it cannot be totally excluded that MNs of M55 and M56 on the same side of the PTG are coupled.

Further input to neck muscle motoneurones after mechanical stimulation of the eye

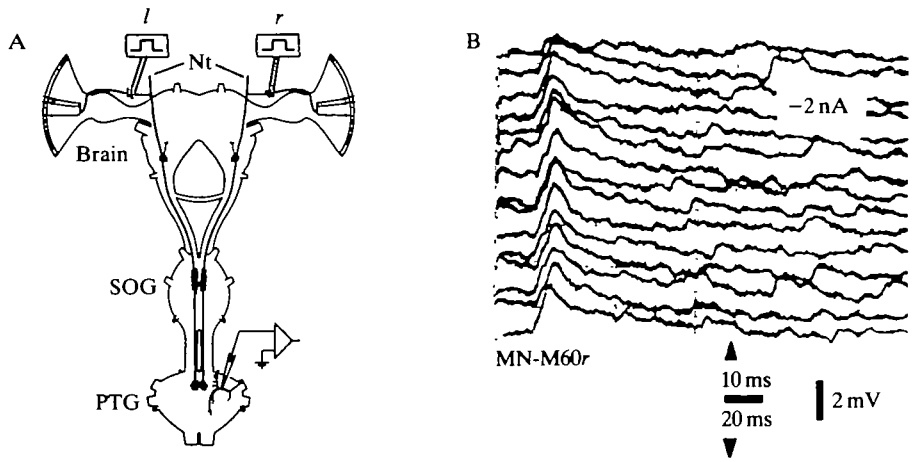
Mechanical stimulation of the eye could release the motor programme of eye cleaning or of another behaviour pattern, such as avoidance or an aggressive act against the stimulation tool, and sometimes the animal did not react to the stimulus at all. In all cases the MNs of M55, M56 and M60 on either side were inhibited with different strengths throughout the stimulation period, from the onset of the stimulation until its end or until a movement started (Fig. 7A,B, the time of stimulation is marked by a black bar). The inhibition disappeared when the neck connective on the side of the recorded MN was cut (Fig. 7C). Both the short latency inhibitory input to

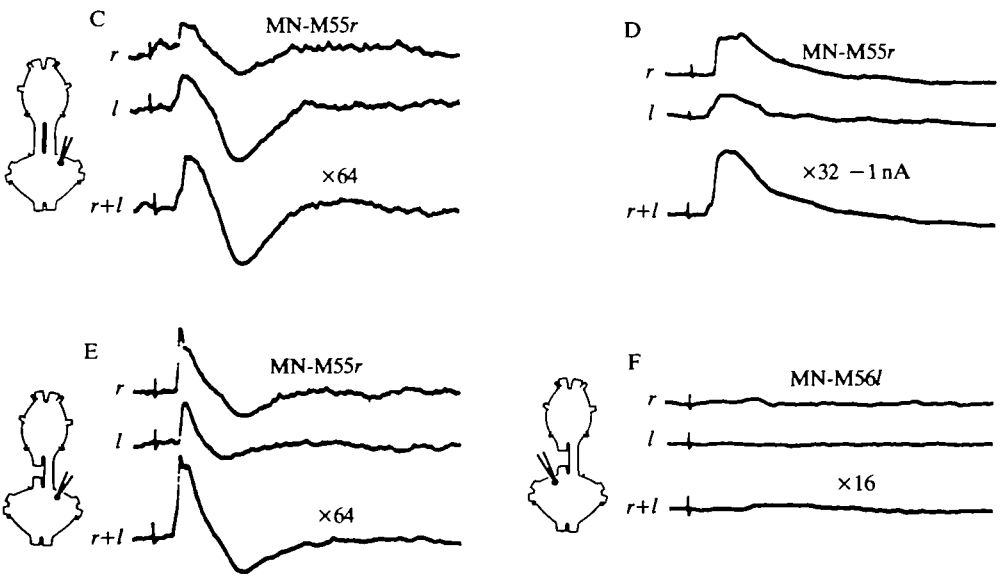
Fig. 9. Intracellularly recorded responses of neck muscle motoneurones (MN) to single electric shocks applied to the axons of interommatidial mechanoreceptors. (A) Experimental situation: schematic outline of brain, suboesophageal ganglion (SOG) and prothoracic ganglion (PTG) and the courses of the primary projections of the interommatidial receptors (after Honegger, 1977). Stimulation electrodes were mounted onto the eye branches of both nervus tegumentarius dorsalis (Nt) which contain the axons (see also Methods). (B) Recording of an MN of the right muscle 60 during stimulation (1 Hz, stimuli at the beginning of the traces) on the right side. Each one of subsequent stimuli evoked an EPSP with a latency of about 10 ms. The MN was hyperpolarized with 2 nA to prevent spiking and to enhance the EPSPs. (C–F) Either the right (upper traces), the left (middle traces) or both nerves simultaneously (lower traces) were stimulated at 1 Hz (note stimulus artifacts). The responses of MNs were averaged (16, 32 or 64 events). (C), (D), (E) The same MN of the right muscle 55. Insets show the side of recording and in E and F the side where one neck connective was cut. (C) Intact animal: every stimulus on either side evoked an EPSP followed by an IPSP. (D) Same as C but the MN was hyperpolarized with 1 nA to enhance the EPSPs. Every EPSP consisted of at least two components. (E) Neck connective cut contralateral to the MN recorded from. Same response as in C. The peaks on the EPSPs were caused by spikes which were currently riding on them. (F) MN of the left muscle 56, on the side on which the connective was cut. PSPs were no longer evoked. Abbreviations and insets as in Fig. 5.

possibly all neck muscle MNs of both sides and the longer latency motor programme must be mediated by interneurones, because the sensory projections and the MNs do not have dendritic contacts (Honegger, 1977; Honegger *et al.* 1984).

Electrical stimulation of primary afferents

In the above experiments the interommatidial mechanoreceptors were stimulated by brushing the eye with an eyelash. This did not allow a determination of the exact timing of the stimulus because a different number of receptors was excited at different times with different strengths. For the investigation of the stimulation-coupled inhibition of the MNs which was described in the previous section, electrical





stimuli were applied to the nerve which contains the axons of the interommatidial mechanoreceptors (Fig. 9A). These stimuli excited a number of receptor axons at the same time.

Effects of single shocks

Every stimulus on either side elicited an EPSP with a latency of about 10 ms followed by an IPSP, in all MNs of M55, M56 and M60 from which recordings were made (example in Fig. 9C, two upper traces). Both EPSP and IPSP occurred at the same stimulus threshold and did not change in shape when the stimulus strength was increased. When both sides were stimulated simultaneously, both the EPSP and the IPSP summed (Fig. 9C, lower trace). This suggests separate channels for both sides which converge onto the MNs.

To enhance the EPSPs and to prevent spiking a small hyperpolarizing current was injected into the MNs (Fig. 9B,D). This abolished the IPSPs (cf. Fig. 3) and revealed that the EPSPs were composed of at least two components, as indicated by steps in the rising phases. EPSPs evoked by subsequent stimuli were not constant in amplitude (Fig. 9B). The mean amplitudes of EPSPs and IPSPs varied between 1 and 3 mV when no current was injected into the cells. The different mean amplitudes after stimulation ipsi- or contralateral to the recorded MN were not consistent (compare upper and middle traces in Fig. 9C,D).

When one neck connective was cut the responses of the MNs on the intact side were not changed under either stimulus condition (Fig. 9E). However, in MNs on the operated side PSPs could no longer be evoked (Fig. 9F). Therefore, the information from the sensory systems of both sides must be transferred through the neck connective ipsilateral to the respective MN. At least for stimuli on the contralateral side this must happen *via* interganglionic interneurones.

Effects of stimulus trains

During stimulation of one or both sensory nerves with trains of different frequency, above a certain frequency, all recorded MNs of M55, M56 and M60 were inhibited in proportion to the stimulation frequency (Fig. 10). Probably due to the summation of IPSPs the inhibition outlasted the stimulus train for up to several 100 ms. Longer stimulus trains than those applied in Fig. 10 could elicit eye cleaning behaviour which was apparently normal (the duration depended on the motivation of the animal).

DISCUSSION

Applicability of the preparation

The present study introduces a preparation which allows stable intracellular recordings from prothoracic neurones of crickets which can move in a quite normal way although the prothorax is fixed and dorsally opened. Additionally, lesions can be made (e.g. severing connectives) without losing a penetrated neurone.

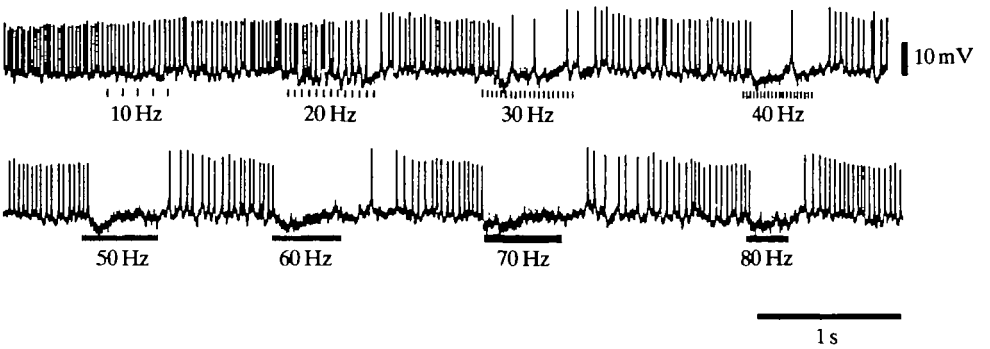


Fig. 10. Intracellular recording from the single excitatory motoneurone (MN) of the right muscle 60 during electrical stimulation of the ipsilateral sensory nerve (cf. Fig. 9A), with stimulus trains of different frequency. Single stimuli are indicated below the recordings. The MN was artificially excited by injection of depolarizing current to visualize inhibition.

In this preparation, with its reduced set of neck muscles, a basically normal eye cleaning motor pattern can be elicited. The discharge patterns in the excitatory MNs of the prominent dorso-ventral neck muscles M55, M56 and M60 (Figs 5–8) were found to be quite similar to those recorded extracellularly in motor nerves of intact animals (Hensler & Honegger, 1985). Therefore the preparation can serve as a model system for investigating the central nervous processes underlying head movements during eye cleaning and most probably during other behaviour. Head movements are involved in nearly every behavioural context and it would be interesting to see how the system switches from one behaviour to another.

Control of motoneurones during eye cleaning

In spite of the removal of the longitudinal neck muscles, head rolls were still possible. They therefore must be driven mainly by dorso-ventral neck muscles, as was confirmed by artificial excitation of single MNs (Fig. 4). In the dissected crickets, the basic discharge patterns in the MNs of these muscles were similar to those in intact ones. However, the mean discharge frequency in M60 was reduced by about 30%. Furthermore, when one neck connective was cut, the MN of M60 contralateral to the side of eye cleaning no longer received relevant input (Fig. 5D), whereas during the same experiment in otherwise intact animals it discharged with a frequency of 18% of that in totally intact crickets (Hensler & Honegger, 1985). In both cases the reduction might be due to partial deafferentation caused by the dissection (cf. Bentley, 1969; Kien, 1979; Robertson & Pearson, 1982; Weeks & Truman, 1984).

Previous to the motor activity of eye cleaning, all MNs of M55, M56 and M60 appeared to be inhibited immediately after the onset of the mechanical stimulation of the eye (Fig. 7A,B). This inhibition could be mimicked by repeated electrical stimulation of the axons of the interommatidial sensilla, whereby summating IPSPs dominated over summating EPSPs (Fig. 10). Therefore, the fast connection between the mechanosensory system of the eye and the neck muscle MNs (Fig. 9) may

be involved in the above mentioned post-stimulus inhibition of MNs. The task of this inhibition could be to support the evaluation of external stimuli by reducing active movements and therefore proprioreceptive input.

During eye cleaning, MNs in both hemiganglia of the PTG received excitatory and/or inhibitory input which was mostly modulated during legstroke-coupled oscillations of the head (Figs 5–8). Thus, the discharge pattern during eye cleaning is not only the differential excitation of MNs, but also their differential inhibition. Part of the cyclic modulation of the MN activity might be due to re-afference when the leg brushes the surface of the eye. However, weak modulation of the activity of M60 can also occur when the foreleg is amputated (cf. Hensler & Honegger, 1985). This indicates that another mechanism must be involved in the generation of this modulation, either a central one, or *via* proprioreceptors in the prothorax, which are driven by the coxa-stump.

In the case of the M60-MN, which released only a few spikes during eye cleaning on its ipsilateral side (Figs 5A,B, 8A), strong inhibition masked a concurrently occurring excitation (Fig. 8A,B). The neuronal basis of this excitation may have an important function within the neural circuit of eye cleaning and the excitation produced in the M60-MN may be important during another behavioural context, but during eye cleaning the excitation appeared to be useless as it was compensated by the inhibition (Figs 5A,B, 8B). Such phenomena may be common and may complicate the understanding of a neural circuit. Therefore, it is advantageous to examine the function of connections between neurones in behaving animals to establish their relevance in a circuit (cf. Heitler, 1983).

Different EPSP amplitudes and the steep rising phases of both the EPSPs and the IPSPs indicated that the input to the MNs originated from a number of spiking neurones. A similar organization was found for the input to flight MNs of the locust (Robertson & Pearson, 1982; Hedwig & Pearson, 1984). It remains to be investigated whether input from non-spiking interneurones (e.g. Burrows, 1981) also plays a role.

Lesions of one neck connective revealed that prothoracic MNs are at least partially controlled *via* intersegmental interneurones (INs) which project through the neck-connective contralateral to the side of eye cleaning, i.e. the sensory input eliciting it (Figs 5D, 7C, 8B). Whether the INs which are primarily responsible for that are descending or ascending or both remains to be investigated. At the moment some ascending INs are known which appear to participate in the formation of the motor output (K. Hensler, in preparation).

In summary, the motor programme for eye cleaning must be generated by a complex network of INs of the SOG and the PTG, which involves both neck connectives (see also Hensler & Honegger, 1985).

Properties of neck muscle motoneurones

In quiescent animals all neck muscle MNs recorded from received a steady input of IPSPs (Fig. 3). Bentley (1969) observed the same in mesothoracic MNs of deafferented crickets and Kien (1979) reported a steady barrage of PSPs to neck

muscle MNs of the locust (not reported whether these were IPSPs or EPSPs). In contrast, Burrows (1975) did not find such an input to leg and flight MNs of the locust. The function of these IPSPs is not quite clear. They may control the general excitability of MNs or, as Bentley (1969) suggested, MNs might be excited by the suppression of a steady inhibition.

When stimulated artificially *via* injected current, single MNs of M55 and M56 caused a head roll of variable strength to their ipsilateral side, and the single excitatory MN of M60 caused a weak head roll to its contralateral side (Fig. 4). This reflects exactly the discharge patterns during the head rolls of eye cleaning. However, no single MN was able to rotate the head as fast as it occurred during normal behaviour (compare the slope of the recording of head roll in Fig. 4 with those in Figs 5–8; note the different time scales; see also Hensler & Honegger, 1985). When a single MN was stimulated it took 200–300 ms before 90 % of the respective final head roll angle was reached. This is the same time range as needed for other insect muscles to reach their maximum contraction (Bennet-Clark, 1975; Phillips, 1980). The need for fast head movements may partially explain the mass of neck muscles and MNs.

When current injection into the MNs was stopped, for some MNs of M55 and M56 a certain head deflection was maintained without MN activity. Similarly it has been observed that after voluntary head movements of eye cleaning, the head could stay partially deflected without neural activity (Hensler & Honegger, 1985). Since the maintained deflection occurred only after stimulation of some MNs (Fig. 4A,B) and the head of an anaesthetized cricket returned to the normal position when it was passively rolled, it appears likely that the partial deflection results from the resting tension of neck muscles (Adams & O'Shea, 1983; Hoyle, 1983).

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