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Motor output characterizing thanatosis in the cricket *Gryllus bimaculatus*

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Summary

The cricket *Gryllus bimaculatus* displays a sudden rigid immobility (thanatosis) when voluntary leg movements are forcibly restrained. The tibial joints in all legs are stiffly immobilized for several minutes. The flexed-leg posture typical of thanatosis is maintained by the flexor tibiae muscle. To characterize thanatosis at the motor output level, the mechanical and physiological properties of the metathoracic tibia muscle were investigated. The accessory flexor muscle, especially well-developed in the cricket, acts to stably maintain the tibial flexion driven by the main flexor muscle. Extracellular recordings from the flexor muscle of tethered crickets revealed that activity of intermediate- and fast-excitatory units was almost completely suppressed, while slow-excitatory units persisted in firing tonically during thanatosis. The firing rate of slow-excitatory units progressively increased as the tibia flexed, but remained less than the firing rate seen in the quiescent state. Common inhibitory motor neurones that fire sporadically in the quiescent state were suppressed during thanatosis, especially in the beginning, and showed a large excitation immediately after arousal. These findings suggest that the entire motor neuronal pool is held under active suppression during thanatosis, and that flexor muscle rigidity is maintained by a weak discharge of the slow exciters together with suppression of the inhibitors.

Key words: cricket, *Gryllus bimaculatus*, common inhibitory motor neurone, accessory flexor, tonic immobility.

Introduction

Death-feigning (thanatosis) is a tonic immobility found in many invertebrate and vertebrate orders (Fabre, 1910; Rabaud, 1919; Steiniger, 1936). In arthropods this state is usually induced reflexively by a mechanical disturbance, such as a gentle grasp of the thorax (Steiniger, 1936; Nishino and Sakai, 1996; Faisal and Matheson, 2001) or restraint of the legs (Steiniger, 1936; Nishino et al., 1999). Bullock and Horridge (1968) roughly classified the tonic immobility found in arthropods into three kinds: (a) resting of camouflaged forms, (b) sudden freezing or pose when startled, and (c) the cataleptic state, or immobility maintained for many minutes. They noted that in the first two states, the animal resumes activity after a mechanical disturbance, but in the third it is rigid even if cut into pieces. The cricket *Gryllus bimaculatus* freezes suddenly upon restraint of the legs, maintains the restrained posture for ca. 3–4 min and arouses abruptly. Arousal also follows a strong mechanical disturbance such as poking or prodding, although behavioural responses to visual, auditory and weak tactile stimuli are strongly suppressed (Nishino and Sakai, 1996). Thanatosis in the cricket therefore appears to be in the second of the categories above (Nishino and Sakai, 1996).

Thanatosis in animals has been behaviourally characterized by (1) suppression of the righting response (Steiniger, 1936; Godden, 1972; Nishino and Sakai, 1996; Faisal and Matheson, 2001), (2) maintenance of an unusual posture or of an unusual posture passively taken (Steiniger, 1936; Godden, 1974; Bässler, 1982; Nishino and Sakai, 1996). The latter condition in (2) is termed ‘catalepsy’, in which leg joint reflexes are predominantly velocity-sensitive but not position-sensitive (e.g. Bässler and Foth, 1982; Driesang and Büschges, 1993; Wolf et al., 2001).

As thanatosis occurs in many postures due to its cataleptic nature, one might ask what physiological characteristics distinguish the thanatotic state from the normal quiescent resting states. Physiological differences have been indicated by detailed behavioural studies. For example, during thanatosis in orthopteran insects, all movements of the body (including ventilations in the abdomen) and appendages are strongly suppressed and a rigid posture is maintained (Nishino and Sakai, 1996). Most strikingly, the leg joints appear to be stiff, not flaccid, and complete immobilization may continue for 10–20 min (Hoyle and Field, 1983a; Nishino and Sakai, 1996). In contrast, the voluntary resting state differs from the cataleptic state because loss of muscle tonus (antennal and neck inclination) is prominent in resting crickets (Nishino and Sakai, 1996) and honeybees (Kaiser, 1988; Sauer et al., 2003). This immobility is frequently interrupted by short bouts of

locomotor activity and by limb or antennal movements (Kaiser, 1988; Nishino and Sakai, 1996; Sauer et al., 2003). These findings collectively indicate that during thanatosis the skeletal muscles exhibit persistent rigidity but also plasticity when forcibly stretched. However, no clear motor output characterizing the thanatotic state has yet been defined.

Physiological studies focusing on peripheral motor control have revealed two different mechanisms for maintaining persistent tonic immobility. A primitive orthopteran, the weta *Hemideina femorata,* displays a defensive posture with the metathoracic tibiae fully extended, maintaining this posture for several minutes without any electrical activity in the extensor tibiae muscle (Hoyle and Field, 1983a). This 'catch-like tension' is triggered by a brief spike burst from the excitatory motor neurones immediately after octopamine is released from neuromodulatory, dorsal unpaired median (DUM) neurones (Hoyle and Field, 1983b). Similar catch-like tension (i.e. prolonged maintenance of residual tension) has been found in the claw opener muscle of the crayfish, *Astacus* sp. (Hawkins and Bruner, 1979) and the metacoxal muscle of the cockroach, *Periplaneta americana* (Chesler and Fourtner, 1981).

In the stick insect *Cuniculina impigra* the thanatotic display is maintained by the continuous activation of slow excitatory motor neurones. In response to visual stimulation or to mechanical disturbance all six tibiae are fully extended to form the stick posture. Although fast motor units are reflexively activated by the tibial displacement, this does not cause arousal (Godden, 1972). Thanatosis occurs naturally during daylight and alternates to the camouflaging resting posture, in which the tibiae are more flexed. The motor neuronal activity is fundamentally similar in the two states except that the firing rate of the slow extensor tibiae (SETi) is higher in the thanatotic state in order to maintain the fuller tibial extension seen in the stick posture (Godden, 1972; Bässler, 1982).

In contrast, the cricket is an active walker in which the behavioural switching from arousal to thanatosis, and *vice versa*, occurs rapidly. This allows a reliable correlation between neural events and behaviour. A preliminary study has shown that the thanatotic posture is maintained by continuously active slow motor units, as in stick insects (Nishino et al., 1999).

To determine the motor output typical of thanatosis, I have focussed on the anatomy and physiology of the metathoracic flexor tibiae. This is one of the principal posture-controlling muscles and is fundamental to the maintenance of tibial flexion during thanatosis. Its motor innervation has been extensively studied in locusts (Hoyle and Burrows, 1973; Phillips, 1980; Sasaki and Burrows, 1998), katydids (Theophilidis and Dimitriadis, 1990), tree wetas (O'Brien and Field, 2001) and crickets (Nishino, 2003). Electrical recordings from the flexor motor neurones in minimally restrained crickets revealed that stable tibial flexion depends on both mechanical and physiological factors. The elaborated accessory flexor muscle stabilizes the femoro–tibial (F–T) joint, with the activity of the common inhibitory motor neurones (CIs) being suppressed to maintain the muscle tonus created by the activity of the slow excitatory motor neurones.

Materials and methods

Animals

Adult male crickets *Gryllus bimaculatus* DeGeer from breeding colonies at the Okayama University and Hokkaido University were used in all experiments. The results are derived from more than 90 crickets.

Behavioural experiments

Thanatosis can be induced in either the ventral-up position or the dorsal-up body position. During thanatosis, the metathoracic F–T joint can be immobilized at any angle between 0° and 100°, corresponding to the range in which the flexor tibiae muscle normally functions (Nishino et al., 1999). To standardise the experiments, only 'flexed-leg thanatosis' (termed in Nishino et al., 1999) was studied. This was induced by pressing both sides of the pronotum and forelegs gently for 3–5 s with the thumb and forefinger, whereupon the cricket enters thanatosis with all legs flexed (Fig. 1A; Nishino and Sakai, 1996). The cricket was gently released and placed on a flat wooden bar, 2 cm wide, with its ventral side up to eliminate local reflexes caused by contact of the legs with the substrate (Fig. 1A). The switch from thanatosis to arousal was indicated by the righting response. The bodily movements during thanatosis were monitored with a photo-coupler placed beside the abdomen (P, Fig. 1A). The voluntary movements of animals were monitored verbally on the voice channel of a data recorder or by a video camera (Handy-Cam, Sony, Tokyo, Japan).

Ablations were made through a door-like incision in the cuticle over the region of interest, avoiding complete removal of the cuticle. The dissection procedures were adapted from Nishino and Sakai (1996) and Nishino et al. (1999). The single motor nerve branches supplying the flexor tibiae muscles in the metathoracic legs were cut where they exited the main leg nerves. As for the proximal flexor nerve and the accessory flexor nerve (described in Results), either the anterior or the posterior branch was cut to minimize damage to the muscle fibres. After an operation, the opened flap of cuticle was closed to its initial position. About 8 h after an operation, the femoro–tibial joint angle (F–T joint angle) during flexed-leg thanatosis was measured manually using a small protractor (Nishino et al., 1999). The flexor muscle tonus was then determined from the strength of the resistance reflex, as follows. The cricket, still in thanatosis, was hung from a horizontal wire by the tarsal claws of the metathoracic legs and the F–T joint angle measured again. This posture is known as 'catalepsy during hanging' (Nishino and Sakai, 1996; Nishino et al., 1999). In intact crickets, the body mass caused the F–T joints to open from full flexion (0°) to 30–40°, which was maintained until arousal (Fig. 1B). After the experiment, conventional forward-filling (see below) from the main leg nerve was carried out to ensure that the intended nerve had been cut. The difference between the operated and intact

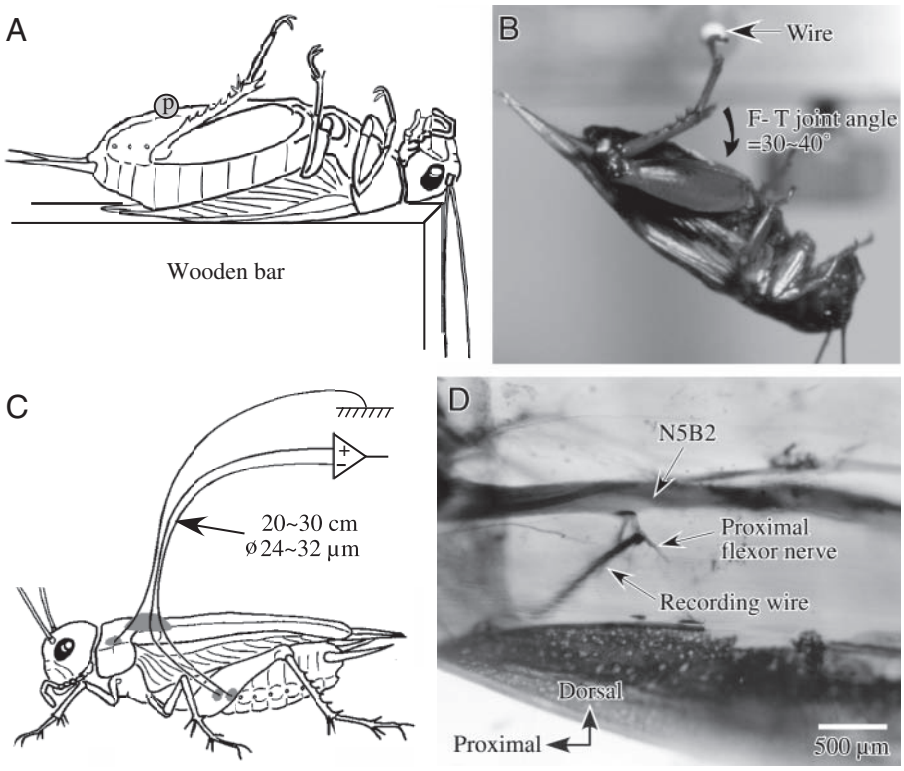

Fig. 1. (A) Cricket in flexed-leg thanatosis placed on a flat wooden bar. See Materials and methods for induction procedure. The bodily movement during thanatosis was monitored with a photo-coupler (P) settled beside the abdomen. (B) Catalepsy during hanging. The cricket continued to be immobile with the femoro–tibial (F–T) joint opened (30–40°) by the body mass until arousal. (C) Set-up for extracellular recording in a minimally restrained cricket. A pair of insulated copper wires were inserted through small holes on the cuticle to the flexor tibiae muscle and are bound with an earth electrode extended from the pronotum and fixed on the forewings. Each insertion point of the wire was fixed by wax resin (grey). (D) The recording site, revealed by a forward-fill of the main leg nerve, nerve 5B2 (N5B2). The recording electrode remained inserted. The leg was cleared with methyl salicylate. This preparation, in which the tip of the recording wire contacts with the proximal flexor nerve, was used for Fig. 4A.

groups was assessed using the Student's *t*-test ($P<0.05$; see Table 1 for sample sizes).

Anatomy

The peripheral motor nerves were stained by forward-fills from the main leg nerve (N5B2) while motor neuronal somata and dendrites in the metathoracic ganglion were stained by back-fills from the peripheral motor nerves. The dissection and staining procedures were adapted from Nishino (2003). 6% solution of nickel chloride hexahydrate (Merck) and 1% solution of fluorescent dyes such as dextran, tetramethyl rhodamine (MW=3000, Molecular Probes, Eugene, OR, USA) and dextran, fluorescein (MW=3000, Molecular Probes) were used as marker substances. The nickel-filled specimens were reacted with rubeanic acid to precipitate the nickel. The ganglia were fixed in Alcoholic Bouin and the peripheral tissues in 5% neutral formaldehyde solution before being dehydrated through an ethanol series, cleared in methyl salicylate and photographed under a light microscope. The specimens were further processed by silver intensification if necessary (Bacon and Altman, 1977). Fluorescent dye-filled specimens were fixed in 4% neutral formaldehyde solution, dehydrated through an ethanol series, and viewed under a confocal microscope (LSM510, Zeiss, Jena, Germany). Optical sections (thickness: 5 µm each) were

Table 1. *Effects of ablation of motor nerve branch, or muscle tissue in the metathoracic leg, on femoro–tibial joint angles maintained during thanatosis and catalepsy*

	F–T joint angle (deg.)	
Treatment	Thanatosis during lying	Catalepsy during hanging
Intact (*N*=60)	0	33±11
Middle flexor nerve-cut (*N*=48)	0	31±4
Distal flexor nerve-cut (*N*=60)	0	31±6
Posterior branch of proximal flexor nerve-cut (*N*=45)	2±4*	45±14*
Posterior branch of accessory flexor nerve-cut (*N*=60)	2±2*	87±14*
Muscle bundles innervated by posterior branch of accessory flexor nerve-removed (*N*=36)	15±11*	102±12*
Flexor apodeme-cut (*N*=60)	89±5*	155±6*

F–T, femoro–tibial. For details of treatment, see Materials and methods.
Values are means ± S.D. for the F–T joint angle under each condition. *N*=total sample size (three measurements/cricket in each treatment).
*Statistically significant ($P<0.05$, Student's *t*-test) with respect to the intact group.

reconstructed two-dimensionally using commercial software linked to the LSM.

Electrophysiology

To identify the physiological types of neurones sending axons into the flexor nerve branches and to see their neural activity during thanatosis, quiescence and walking behaviour, extracellular recordings were made from both restrained and freely moving animals tethered by fine recording leads.

For recording under restrained conditions, the cricket was anaesthetized on iced water for 10 min and then fixed ventral-side-up on a chamber filled with beeswax. The dorsal halves of the body and pro- and mesothoracic legs and the posterior halves of the metathoracic legs were embedded in Plasticene™ to prevent voluntary movements. The metathoracic F–T joint to be recorded was fixed at about 80°. The gut was removed *via* an incision on the dorsal tip of the abdomen to eliminate body hemolymph, which disturbs the observation of the recording sites due to its coagulation. To expose the accessory flexor nerve the anterior cuticle covering the distal femur and also the anterior muscle bundles of the accessory flexor were removed. The cavity formed by removal of the muscle tissue was filled with cricket saline (Nishino and Sakai, 1997). Care was taken not to cut the apodeme of the femoral chordotonal organ (FCO) running very close to the nerve as cutting the apodeme causes a severe reduction in the activity of slow excitatory motor neurones. The accessory flexor nerve was cut proximally to the muscle and efferent activity recorded from its proximal cut-end with a suction electrode. In several recordings, the efferent activity was simultaneously recorded from the proximal cut-end of N5B2 in the distal-end of the femur using a suction electrode. Intracellular muscle recordings were made from the middle of the accessory flexor muscle fibres using a borosilicate glass electrode filled with 4 mol l^{-1} potassium acetate to give a tip resistance of 10–13 MΩ.

For recordings under tethered conditions, crickets anaesthetized with carbon dioxide were fixed onto the beeswax plate with stapler pins, leaving all body appendages intact. To record from one of the three main flexor motor nerves, a pair of copper electrodes (insulated except for the tip, 32 μm in diameter) was bound with fingernail lacquer and inserted through small holes in the cuticle (Fig. 1C). To record motor neuronal activity as directly as possible, the recording electrode was adjusted to obtain clear extracellular records from the targeted nerve. The reference electrode was placed on the surface of the muscle compartment innervated by that nerve. Each electrode was fixed in place with a wax-resin mixture. The cricket was placed in a plastic-walled observation arena (20 cm×20 cm×10 cm). The pair of electrodes was bound to an earth electrode inserted through the pronotum, and then all were fixed with wax resin onto the forewings and connected to the head stage of a differential amplifier (Fig. 1C). After the experiment, a conventional forward-fill from N5B2 was carried out, leaving the recording wire inserted, to check the distance between the nerve and the tip of the recording wire (Fig. 1D). Data derived from preparations in which the nerve and the tip of the recording wire were not attached were excluded.

To gain access to the accessory flexor nerve a small door-like incision was made on the posterior surface of the distal femur of the anaesthetized cricket and opened by inserting an insect pin between the cuticle flap and the muscle. The recording electrode (insulated copper wire, diameter: 22 μm) was scratched slightly around the tip to remove the insulation and then inserted through a small hole in the cuticle flap. It was tightly coiled twice around the posterior branch of the accessory flexor nerve and the body haemolymph around the recording site replaced with high vacuum grease (silicon lubricant, Toray Silicone, Tokyo, Japan), ensuring that the electrical activity was recorded only from the accessory flexor nerve. The reference electrode was inserted through another hole made on the cuticle flap and embedded in the silicon grease close to the nerve branch. After the cuticle flap was closed, each electrode was fixed in place with wax resin and then bound with the earth electrode as described above.

Electrical activity was recorded using an amplifier (Iso-DAM8A, WPI, Sarasota, USA) and displayed on an oscilloscope and a data acquisition system (Omniace, NEC, Tokyo, Japan). Data were stored on open reel tapes or DAT cassettes and analysed with the aid of custom-made Lab View v. 6I programs.

Definitions

The main body of the flexor tibiae muscle is arbitrarily named the ‘main flexor muscle’ to distinguish it from the ‘accessory flexor muscle’. As all motor neurones innervating the flexor muscle in the cricket are morphologically similar to those of locusts (Nishino, 2003) the nomenclature of the neurones follows that of the locust (for a review, see Burrows, 1996). Excitatory motor neurones and inhibitory motor neurones are often abbreviated to ‘exciters’ and ‘inhibitors’, respectively. Units of slow, intermediate and fast exciters and inhibitors were discriminated by their spike amplitudes and burst characteristics (Hoyle, 1980; Hustert and Gnatzy, 1995; Tauber and Camhi, 1995; Berkowitz and Laurent, 1996; Nishino et al., 1999). When the amplitudes of two units were similar (e.g. inhibitor *vs* intermediate exciter), spike waveform shape was used as an additional criterion.

During recordings from tethered animals, the deep resting state (termed ‘sleep-like state’; Kaiser, 1988), characterized by antennal and head inclination, was not observed because mechanical disturbances were given to the cricket to induce thanatosis. Instead, brief quiescent episodes maintained from 3 to 30 s appeared between voluntary movements. Accordingly, this immobile state was termed simply the ‘quiescence’ or ‘quiescent state’.

Results

Characteristics of motor neuronal innervation of the flexor tibiae muscle in the cricket

The flexor tibiae muscle in orthopteran insects comprises the

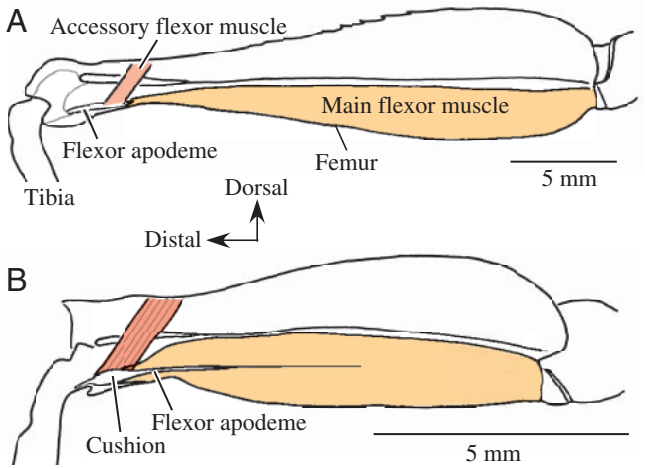

Fig. 2. Camera lucida drawings of the flexor tibiae muscle in the metathoracic leg in the locust *Locusta migratoria* (A) and the cricket *Gryllus bimaculatus* (B). The relative size of the accessory flexor muscle (red) against the main flexor muscle (orange) in the cricket is larger than that in the locust. Whereas the locust has a thin flexor apodeme in the distal femur, the cricket has a cushion enlargement on which the accessory flexor muscle attaches.

main flexor muscle that lies axially in the ventral femur, and the accessory flexor muscle that lies diagonally (45°) in the distal femur, both inserting onto a common flexor apodeme (Fig. 2; Heitler, 1974; Hustert and Gnatzy, 1995). However, different modifications occur in between the metathoracic flexor muscle of the locust, *Locusta migratoria*, and the cricket *Gryllus bimaculatus*. The femur of the locust has a long, slender form (Fig. 2A), while that of the cricket is shorter and less tapered, creating more space dorso-ventrally in the distal femur (Fig. 2B). In the cricket, the cushion on which the accessory flexor muscle attaches is stretched axially from the apodeme (Hustert and Gnatzy, 1995). Accordingly, the accessory flexor muscle of the cricket is relatively larger than that of the locust (volume of fixed, carefully dehydrated samples: 9.4% of the whole flexor muscle volume *vs* 6.3%, respectively, N=2).

The main flexor muscle in the cricket is compartmentalized into three regions, the proximal, middle and distal regions, each innervated by a separate nerve branch diverging from the main leg nerve (N5B2): the proximal flexor nerve, middle flexor nerve and distal flexor nerve, respectively (Fig. 3). The proximal flexor nerve bifurcates at its base to innervate the anterior and posterior muscle bundles (Fig. 3B). This morphologically simple innervation contrasts with the pattern in locusts (Sasaki and Burrows, 1998) and katydids (Theophilidis and Dimitriadis, 1990), in which the homologous muscle is innervated by numerous short branches diverging from N5B2.

The accessory flexor muscle comprises two groups of 5–6 muscle bundles inserting onto the anterior and posterior edges of the cushion. These are innervated by bifurcated branches of the accessory flexor nerve diverging from N5B2 (Fig. 3B).

The four flexor nerve branches carry only efferent neurones, which are excitatory, inhibitory and/or DUM neurones (Nishino, 2003). As in locusts (Hoyle and Burrows, 1973; Hoyle, 1980), the excitatory motor neurones are categorized into slow, intermediate and fast types according to their

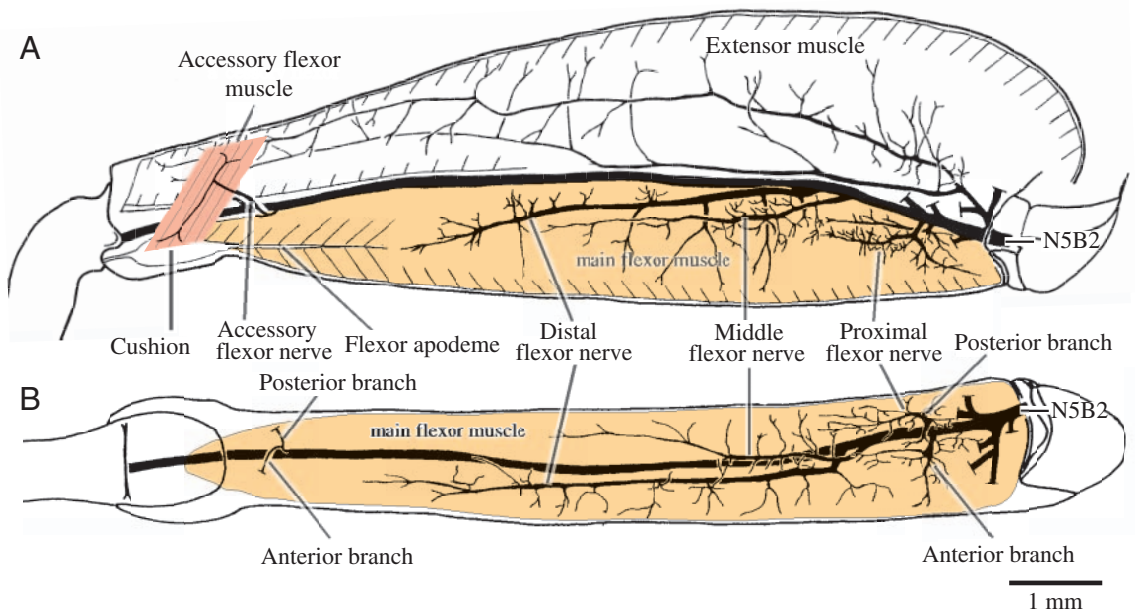

Fig. 3. Camera lucida drawings of motor nerve innervation of the flexor and extensor tibiae muscles in the cricket metathoracic leg, viewed posteriorly (A) and ventrally (B). The main flexor muscle (orange) is divided into the proximal-, middle- and distal regions, which are locally innervated by the proximal flexor nerve, the middle flexor nerve and the distal flexor nerve, respectively. A pair of muscle bundles of the accessory flexor muscle (red) are innervated by the anterior and posterior branches of the accessory flexor nerve diverging from the main leg nerve (N5B2).

physiological properties. The slow-type fires tonically to maintain muscle tonus, while the fast-type fires very briefly but leads to a strong twitch contraction. The intermediate-type fires in longer-lasting bursts with a progressively declining frequency during visible movements (Nishino et al., 1999; Nishino, 2003). Recordings from the motor nerve branches revealed that the proximal flexor nerve has 12 exciters (3–4 slow-, 7–8 intermediate- and 1–2 fast types), the middle flexor nerve has three (two intermediate- and one fast-types), the distal flexor nerve has four (one intermediate- and three fast-types) and the accessory flexor nerve has four (three slow- and one intermediate-types). Thus the main flexor muscle changes progressively from slow to fast innervation distally, while the accessory flexor muscle is largely slow in nature. Two DUM neurones were faintly stained only when the distal flexor nerve was back-filled, suggesting that two thin axons from the DUM neurones supply the distal region of the main flexor muscle (Nishino, 2003).

Differential nerve back-fills revealed that the three muscle compartments of the main flexor muscle are innervated by different axons, except that one intermediate exciter sends axons into both the proximal flexor nerve and the middle flexor nerve. In total 18 exciters innervate the main flexor muscle, the largest number so far reported from orthopteran insects (Nishino, 2003). Overlapping innervation is rather prominent in the muscle compartments that are distant to each other. The proximal compartment of the main flexor muscle and the accessory flexor muscle both receive overlapping-innervation from two exciters and two inhibitors (Nishino, 2003).

Effects of ablation of the motor nerves on maintenance of the thanatotic posture

In order to assess the roles of the different compartments of the flexor muscle in maintaining tibial flexion, ablation experiments of nerves or muscle bundles were performed in both metathoracic legs, and then the flexor muscle tonus was evaluated by measuring the F–T joint angles of the operated legs when the cricket assumed both thanatosis during lying (see Fig. 1A) and catalepsy during hanging (see Fig. 1B). In Table 1, all experimental data are compared to the control F–T joint angles of intact animals: 0° (full flexion) during lying, and 33±11° during hanging. The data are presented in order of increasingly greater disruption.

The legs with the middle flexor nerve-cut or the distal flexor nerve-cut showed no significant differences from intact legs (Table 1). However, the leg flexion response occurred more slowly than in intact legs. Cutting the posterior branch of the proximal flexor nerve resulted in a small but significant loss of muscle tonus: the operated F–T joints were slightly opened compared to intact legs. Cutting the posterior branch of the accessory flexor nerve gave a similar effect to proximal flexor nerve-operated joints during lying. However, the F–T joint was much more open during hanging. Removal of the muscle bundles innervated by the posterior branch of the accessory flexor nerve resulted in more severe deficiencies during both lying and hanging compared to legs with the posterior nerve branch cut. Cutting the anterior branch of the proximal flexor nerve or the accessory flexor nerve produced a similar effect to cutting the posterior branch of the respective nerves (data not shown). Finally, to confirm the role of the flexor muscle in mediating these effects, the flexor apodeme was cut. The F–T joint remained half (lying) or fully open (hanging).

Activity of the motor neurones to the main flexor muscle during thanatosis

Cricket behaviour is disturbed by both chronic restraint and extensive dissection. As thanatosis is seldom observed in these conditions, electrical activity was recorded from free-moving crickets tethered by fine recording leads. Extracellular recordings (neurograms) from the proximal flexor nerve (N=8), middle flexor nerve (N=7) and distal flexor nerve (N=6) revealed that probably all the intermediate and fast exciters were recruited in the induction phase of thanatosis but ceased activity almost completely during thanatosis (Fig. 4A–C). When ventilatory movements occurred frequently during thanatosis, intermediate exciters were likely to be activated more frequently (Fig. 4B), but without apparent coupling to the movements (arrows and time-stretched inset, Fig. 4B). In other recordings from the middle flexor nerve, whereas a barrage of spikes of an intermediate exciter occurred during ventilation in the quiescent state (Fig. 4C), activity of intermediate exciters during ventilation was suppressed during thanatosis (Fig. 4D), Typically, arousal (righting response) was characterized by strong flexor muscle activation with recruitment of fast exciters, as in locusts (Faisal and Matheson, 2001). However, behaviour just after arousal varied: crickets sometimes showed a long quiescence before moving (Fig. 4A), running might begin immediately (Fig. 4B), or slow walking followed a brief quiescence (Fig. 4E). The latter two cases were most commonly observed. Neurogram recordings from tiny axons of slow exciters and inhibitors running through the proximal flexor nerve were extremely difficult, probably because the proximal flexor nerve gives rise to fine terminal arborizations immediately after diverging from N5B2 (see Fig. 3), making these axons too thin to be recorded above the noise level.

Anatomy of the accessory flexor muscle

The above ablation experiments and physiological recordings indicate that flexed-leg posture typical of thanatosis must result from slow muscle activity, controlled by slow exciters and inhibitors. Because the accessory flexor muscle is primarily a slow muscle, it was targeted for neural activity recording. The accessory flexor nerve was isolated from other muscle or nerve tissue before it reached the muscle bundles, where it gave rise to extensive terminal arborizations decorated with rich varicosities (Fig. 5A). A back-fill from the accessory flexor nerve with dextran, tetramethyl rhodamine revealed that two closely bound axons of CIs (these cell bodies were identified in the ganglion) travelled distally through N5B2 to innervate the tarsal levator- and tarsal depressor-muscles (Fig. 5B). Though the posterior branch of the

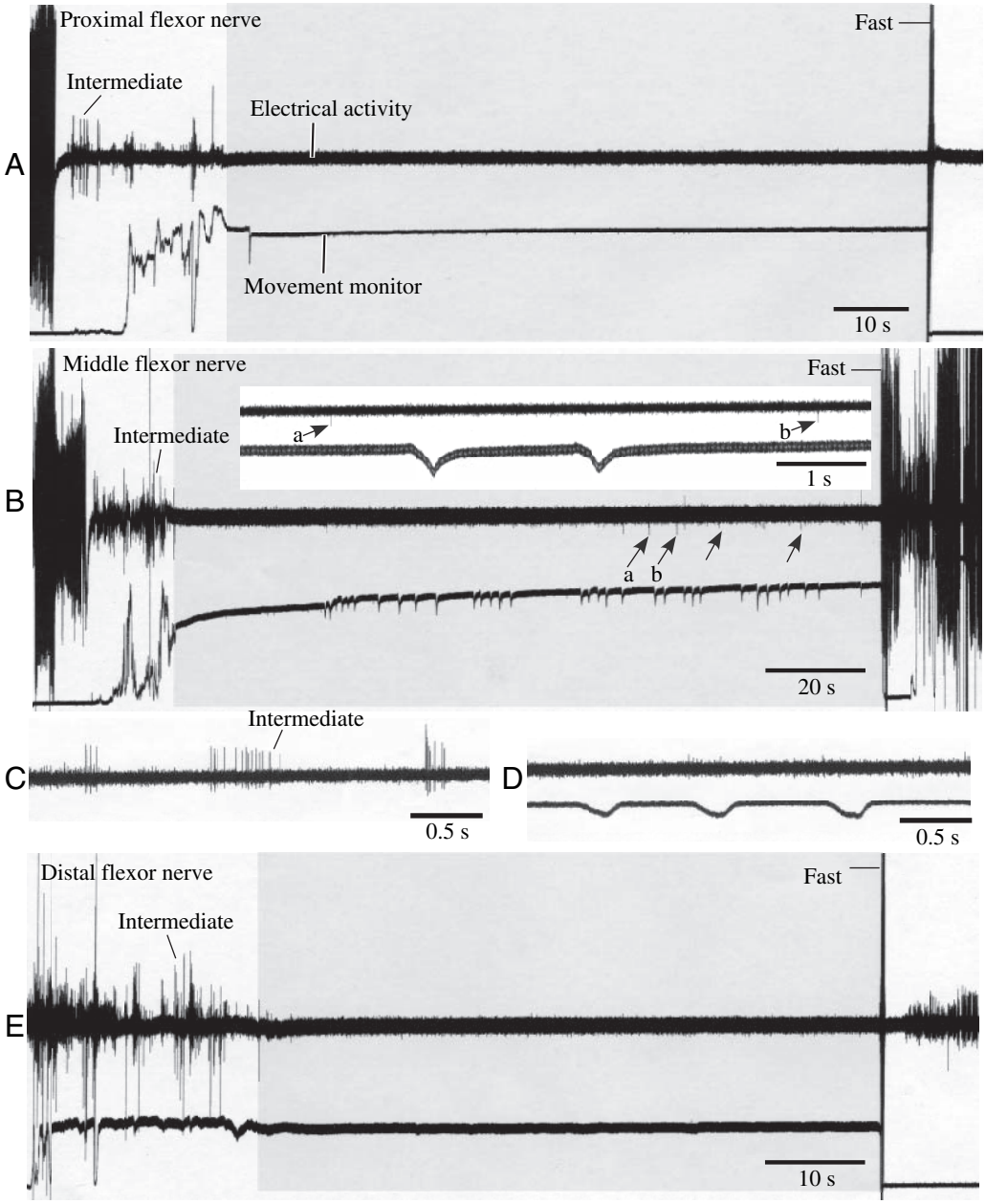

Fig. 4. Motor neuronal activity recorded from the three motor nerve branches (proximal-, middle- and distal-flexor nerves) supplying the main flexor muscle before, during and after thanatosis. The maintenance phase of thanatosis is shaded grey. During thanatosis, activity of intermediate- and fast-exciters was almost completely suppressed (A–E). The intermediate exciters tended to be activated more frequently when ventilatory movements (deflections, lower trace) occurred frequently during thanatosis (B). However, their activity was not necessarily coupled with ventilation phases but seen also in non-ventilatory phases (arrows and time-stretched inset in B). In other recordings, intermediate units vigorously activated with ventilatory movements during the quiescent state (C) were rather suppressed when ventilatory movements occurred during thanatosis (D). Fast exciter units are truncated in A, B and E. Note that voluntary leg movements immediately after arousal occurred in B but not in A and E.

accessory flexor nerve was slightly thicker than the anterior branch (Fig. 5B), back-fills from either branch labelled the identical set and number of motor neurones. A differential back-fill using dextran, tetramethyl rhodamine and dextran, fluorescein labelled neurones sending axons to the accessory flexor nerve (red) and those to the distal flexor nerve (green; Fig. 5C). The somata of all excitatory motor neurones are grouped in the antero-lateral region of the ganglion as in locusts (Burrows and Hoyle, 1972; Phillips, 1980), while those of two moderate-sized CIs are located close to the midline of the ganglion (Fig. 5C). The anterior and posterior CI somata were designated CI2 and CI3, respectively, following the naming of morphological homologues in locusts (Hale and Burrows, 1985; Watson et al., 1985). The somata of the four exciters sending axons to the accessory flexor nerve are much smaller than those sending axons to the distal flexor nerve. No motor neurones with axons in both nerves were detected (Fig. 5C). Another specimen in which the accessory flexor nerve was back-filled with $NiCl_2$ and silver-intensified, showed that there was no consistency in location of the four exciters among individuals whereas the locations of the CIs were almost invariable (compare Fig. 5D with 5C). One exciter (tentative intermediate-type) had a larger soma (Fig. 5C,D) and a prominently thicker axon (arrow in Fig. 5E) compared with the others. The locations of the dendritic arborizations of the exciters and CIs were largely segregated; those of the CIs were more postero-dorsal (outlined by a thin broken line in Fig. 5F) although some overlapping appeared to exist in the dorso-lateral region of the posterior half of the ganglion (Fig. 5F).

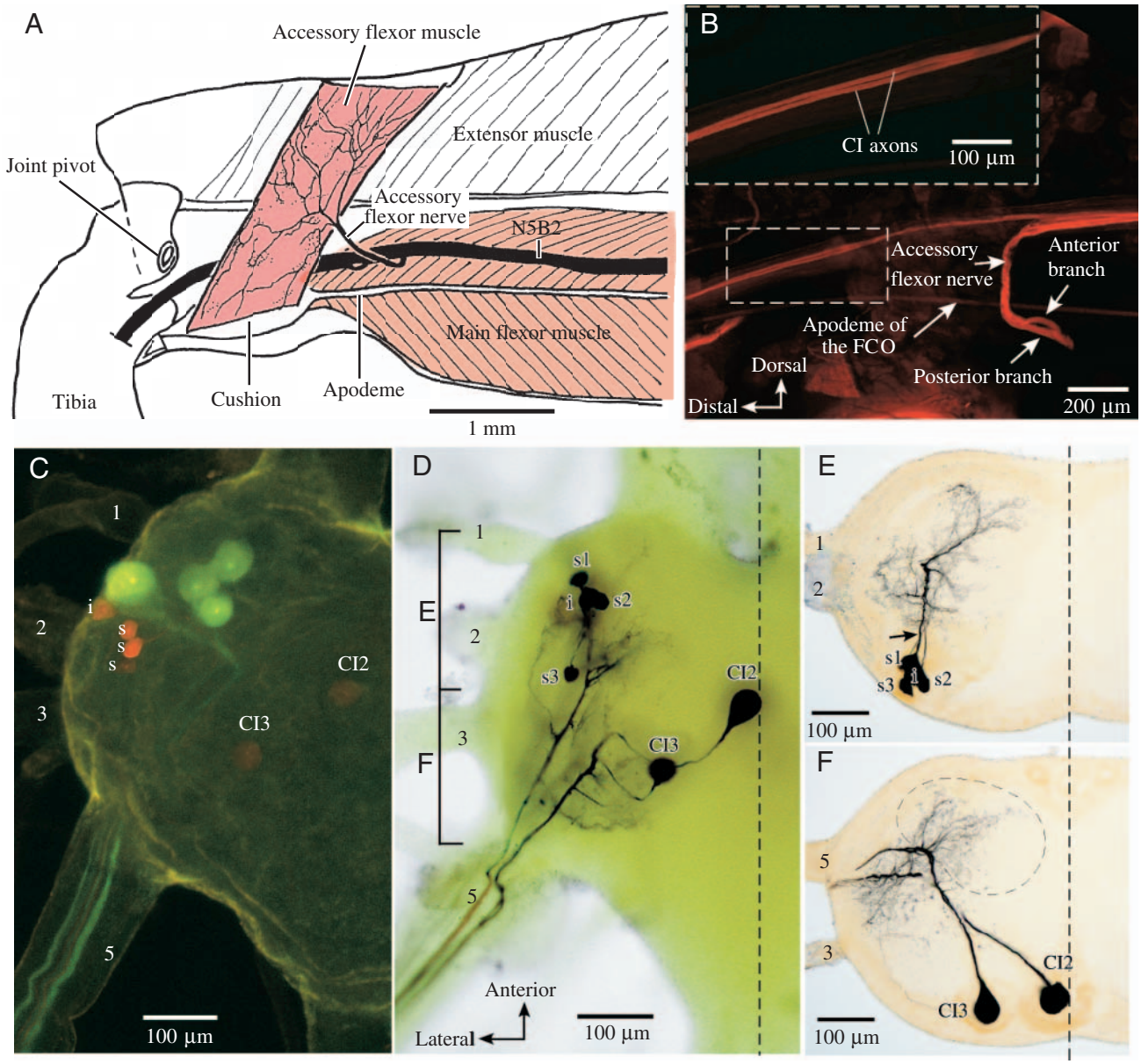

Fig. 5. Anatomy of the accessory flexor muscle and the motor neuronal innervation. (A) Camera lucida drawing of the motor innervation in the distal part of the metathoracic femur, viewed posteriorly. The accessory flexor muscle (red) is inserted diagonally (about 45°) onto the cushion, which is extended from the apodeme on which the main flexor muscle (orange) attaches. The accessory flexor nerve diverged from N5B2 gives rise to fine ramification with rich varicosities in the accessory flexor muscle. (B) Back-fill from the accessory flexor nerve with dextran, tetramethyl rhodamine, revealing that two inhibitor axons (whose somata are identified in the ganglion) pass through N5B2 distally to innervate the tibial muscles (indicated by broken boxes), thus are regarded as common inhibitors. The posterior branch of the accessory flexor nerve is slightly thicker than the anterior branch although both contain identical motor axons. (C) Motor neurones in the metathoracic ganglion, back-filled differentially from the distal flexor nerve and accessory flexor nerve with dextran, fluorescein (green) and dextran, tetramethyl rhodamine (red), respectively. There is no overlap of motor neurones supplying both motor nerves. Somata of four exciters sending axons to the distal flexor nerve (three are fast- and one is intermediate-type) are much larger than those sending axons to the accessory flexor nerve [one tentative intermediate (i) and three slow-type (s)]. Two somata of common inhibitors (CI2, CI3) are located closely to the midline (right edge of photo) and segregate from exciters that congregate in the antero-lateral part of the ganglion. Visible nerve roots are numbered. (D) Motor neurones supplying the accessory flexor nerve in the metathoracic ganglion, back-filled with $NiCl_2$ and silver-intensified. Note that neither sensory afferents nor dorsal unpaired median (DUM) neurones were stained and there is no correlation of soma location of exciters between C and D. Visible nerve roots are numbered and the vertical broken line indicates midline of the ganglion. (E,F) Composite photomicrographs reconstructed from 19 µm transverse sections at the levels indicated in D. Three slow exciters arbitrarily numbered (s1–3), corresponded to those in D. The tentative intermediate exciter (i) is characterized by the larger soma and the thicker axon (arrow in E) compared with slow-type exciters. The primary dendritic area of CIs was circled by thin broken line. Visible nerve roots are numbered and the vertical broken line indicates midline of the ganglion.

Identification and discharge properties of accessory flexor motor neurones in restrained preparations

The number of efferent units recorded from the accessory flexor nerve in restrained, dissected crickets was generally in good agreement with the actual number of efferent axons (Fig. 6). Units recorded from quiescent crickets (Fig. 6A, upper trace) were categorized into three classes. (1) Small units fired tonically at relatively high frequencies (5–20 Hz). Two to three units were usually distinguishable. (2) Two moderate-sized units fired tonically at low frequency, often synchronously. (3) One large unit was silent except for occasional activation with abdominal ventilation. These physiological characteristics indicate that the small units are slow exciters, the moderate-sized units are CIs, and the single large unit is an intermediate exciter.

Several points indicate that the moderate-sized units are CIs. No more than two moderate-sized units were recorded simultaneously from N5B2 at the distal femur (Fig. 6A, lower trace) in which the CIs send axons (see Fig. 5B). Extracellular recordings from the accessory flexor nerve and simultaneous intracellular recordings from the accessory flexor muscle fibres (N=4) show action potentials of the two moderate-sized units corresponding exactly with inhibitory junctional potentials (IJPs) in the muscle (Fig. 6B). The amplitudes of the IJPs markedly decreased as more negative membrane potentials were imposed by current injection. Summation of IJPs was prominent in the muscle fibre when the two CIs fired synchronously (asterisks, Fig. 6B).

The slow exciters and the CIs show recruitment during disturbance stimuli. In Fig. 7A, each of three slow units (indicated by colour in the magnified inset) fired tonically at 6–12 Hz while the smaller unit of two CIs (indicated by 1) fired constantly at 2–5 Hz during quiescent state. When the cricket was slightly disturbed by tapping the substrate, the larger CI (indicated by 2) was recruited synchronously with the smaller unit (Fig. 7B). When the tarsus of the prothoracic leg ipsilateral to the recording site was touched with a wooden stick, both the CIs and the slow exciters were strongly activated and then the discharge gradually declined

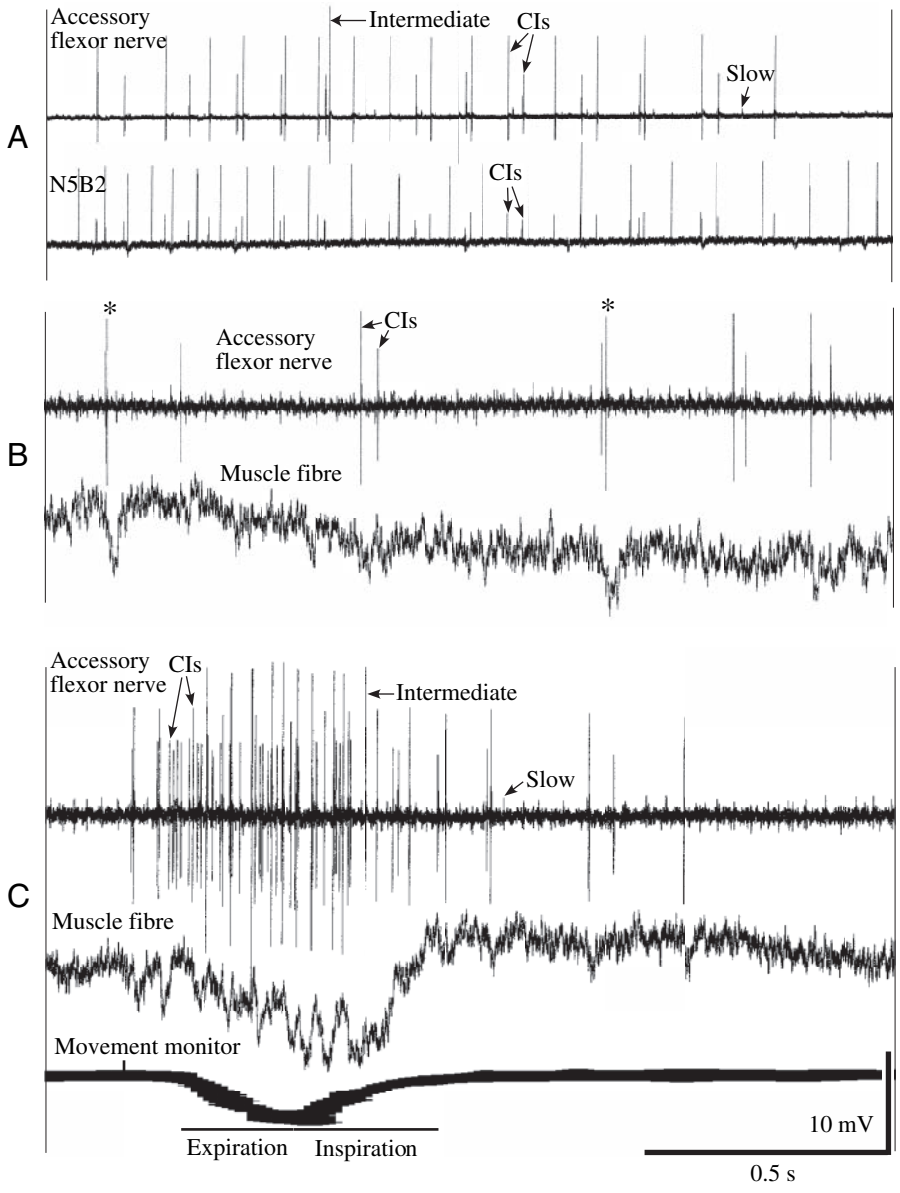

Fig. 6. Physiological characteristics of motor neurones innervating the accessory flexor muscle. (A) Efferent activity simultaneously recorded from accessory flexor nerve and N5B2 in the distal femur when the cricket was disturbed. When two small units, two moderate-sized units, and one large unit fire in the accessory flexor nerve (upper trace), only two moderate-sized units were recorded from N5B2 (lower trace), suggesting that the two moderate-sized units are common inhibitors. (B) Recording from the accessory flexor nerve efference (upper trace) combined with an intracellular recording from a fibre in the accessory flexor muscle (bottom trace) when the cricket is in the quiescent state, showing that spikes of two CIs evoke inhibitory junctional potentials (IJPs) in the muscle fibre. When the two CIs fired synchronously (asterisks), summation of IJPs occurred. (C) Activity pattern of the motor neurones in a ventilation phase. Initially two CIs and subsequently slow and intermediate exciters are activated in expiration, resulting in hyperpolarization of the slow muscle fibre. All units are declined in inspiration, resulting in resumed activity of the muscle potential. Excitatory junctional potentials corresponding to spikes of the intermediate exciter were not seen in this recording but were detected in recordings from other muscle fibres, as noted by Matheson and Field (1995).

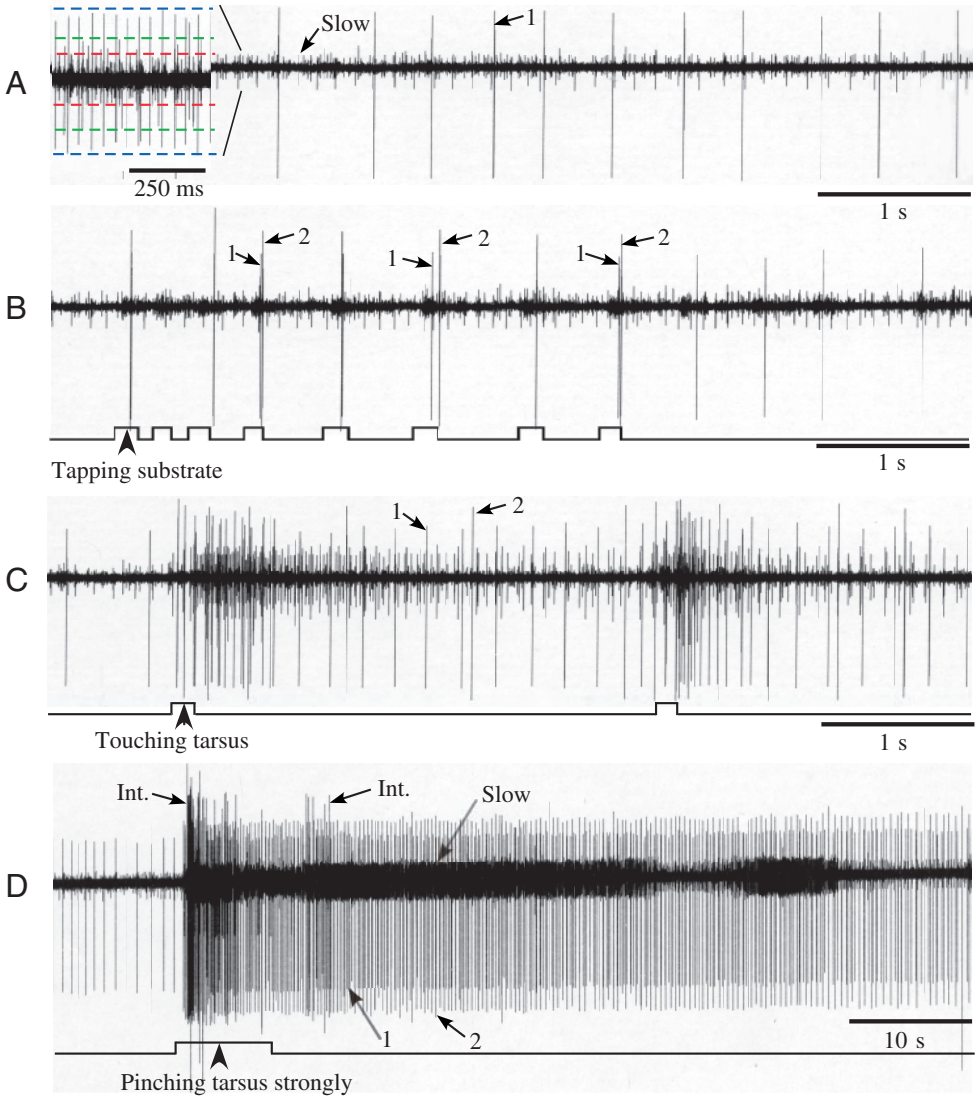

Fig. 7. Effects of external disturbances on activity of motor neurones innervating the accessory flexor muscle. (A) During the quiescent state, three slow units having different amplitudes (coloured in the magnified inset) were clearly discriminated. Only the small CI unit (1) fires tonically at about 3 Hz. (B) Tapping the recording substrate evoked brief activation of the large CI unit (2) additionally to the small CI unit (1) and slow excitatory units (especially in the smallest units). (C) Touching the prothoracic tarsus ipsilateral to the recording site evoked longer activation of two CI units and slow units. (D) Strong pinching of the same tarsus evoked a brief activation of intermediate units and subsequent long-lasting activation in two CIs and slow units. In this example, the tonic activation of CIs and slow units was sustained more than 70 s with slowly adapting discharges.

(Fig. 7C). When the same tarsus was pinched very strongly with forceps (for more than 5 s), the intermediate unit was initially activated but adapted quickly while the slow and the two CIs showed slowly adapting, tonic discharges lasting for more than 1 min (Fig. 7D). The larger CI (2) discharged more slowly than the smaller (1).

As the ventilatory movements are known to influence motor neuronal activity in quiescent locusts (Burns and Usherwood, 1979) and in crickets during thanatosis (Nishino et al., 1999), the fluctuation of activity during ventilation in different types of motor neurones was investigated in detail (Fig. 6C). On the expiration phase of the spontaneous ventilatory movements, the CIs were the first to become active, and subsequently the slow and intermediate exciters were activated in the expiration phase resulting in hyperpolarization of the muscle fibre potential (EJPs caused by the intermediate exciter were not detected in this muscle fibre). On the inspiration phase, this activity quickly waned and the muscle potential returned to the resting level.

Discharge properties of accessory flexor motor neurones in tethered crickets

In all recordings from tethered crickets, units of slow exciters, intermediate exciters and CIs were readily discriminated by their spike amplitudes, firing characteristics and spike shapes (Fig. 8A). However, reliable discrimination of any single units from the three slow exciters or from the two CIs was difficult because their spike amplitudes and shapes were similar and fluctuated.

There was some variation in thanatotic posture from trial to trial in the same cricket and from individual to individual (Nishino and Sakai, 1996). In many cases, a cricket that immediately entered thanatosis with all tibiae flexed exhibited a low ventilation rate and a low responsiveness to mechanical disturbance. However, stimulated crickets occasionally resisted the restraints instead of assuming leg flexion and somehow entered immobility with all F–T joints opened during struggling. In this case, ventilation rates and responsiveness to disturbance more closely resembled those of the quiescent state than those of ordinary thanatosis (Nishino and Sakai, 1996). Thus, thanatosis in which the F–T joints were rigidly flexed was termed ‘strong thanatosis’ and thanatosis in which all F–T joints were loosely opened was termed ‘weak thanatosis’ in this study (Fig. 8B).

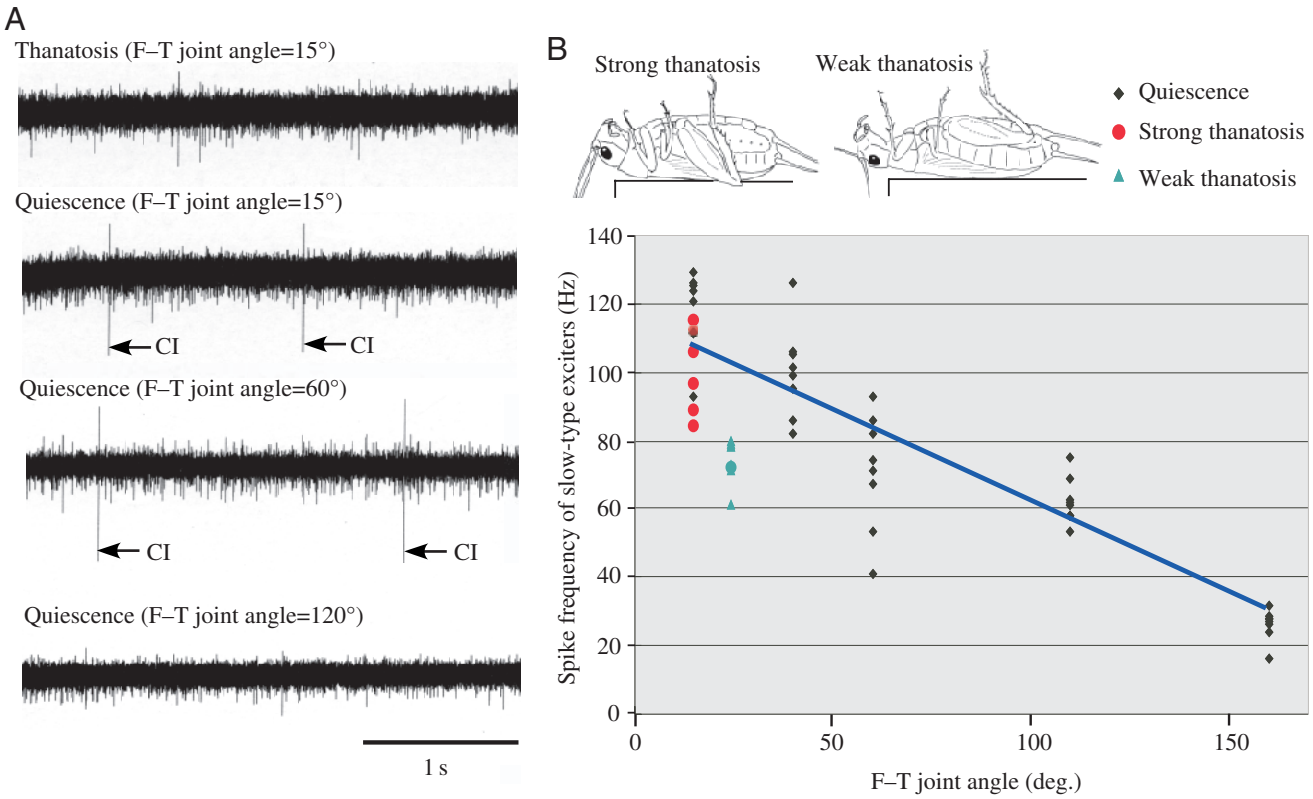

Fig. 8. Activity of slow exciters during immobile postures of the cricket. (A) Raw data of activity of slow exciters when the cricket maintained the recorded F–T joint at various angles during thanatosis or quiescent state. During the quiescent state, at least one CI unit fired sporadically at low frequency (0.5–2 Hz). (B) Average spike frequencies of slow exciters sampled for 5 s when the cricket exhibited strong thanatosis, weak thanatosis (see drawings) or the quiescent state at various F–T joint angles were plotted quantitatively. To count spikes of slow motor neurones, the window (threshold) was set just above the noise level and below the CI spike amplitude. Activity during ventilatory phases and those just after arousal from thanatosis were excluded from sampling. Note that activity of slow exciters during thanatosis is rather suppressed compared with those during the quiescent state at the same F–T joint angle.

Activity of the slow excitatory motor neurones during thanatosis

In all the recordings derived from five individuals, slow exciters were continuously active during both thanatosis and quiescence although their discharge frequencies varied between individuals. In one recording, average frequencies (Hz) of total discharges of slow exciters were measured from 5 s samples when the cricket was in either thanatosis or the quiescent state at various F–T joint angles (Fig. 8A). Sample periods in which ventilatory movements occurred and those just after arousal from thanatosis were excluded from the analysis because slow exciter activity tended to fluctuate in both conditions (see Fig. 6C). When the cricket was quiescent, the slow exciters tended to discharge at progressively higher frequencies as the F–T joint was flexed (although there was fluctuation even if the same F–T joint angle was maintained. Fig. 8B). However, when the cricket was in strong thanatosis (F–T joint angle was slightly opened to 15° due to the operation), the slow exciters fired at a somewhat lower frequency compared to the quiescent cricket at the same F–T joint angle (Fig. 8B). This tendency was more distinct when the cricket was in weak thanatosis with the F–T joint angle at 30°, where the slow exciters maintained a much lower frequency than expected in the quiescent state (Fig. 8B).

Activity of the common inhibitory motor neurones during thanatosis

The CIs show distinctive pattern of activity during and immediately after thanatosis. During strong thanatosis (Fig. 9A), the normal 0.5–2 Hz firing of the CIs during quiescent state (Fig. 8A) was suppressed. Only eight spikes from CIs were identified in 42 s of the maintenance phase of thanatosis (asterisks in Fig. 9A). In contrast, strong CI firing (at about 15 Hz), possibly due to disinhibition, occurred just after arousal, although the cricket was motionless with the recorded F–T joint maintained at 120°. The intermediate exciter, which causes visible leg movements was not recruited. As walking commenced, CI firing diminished rapidly while large motor neurones were recruited (Fig. 9A). On the other hand, during weak thanatosis (F–T joint maintained at 95°), the CIs and the intermediate exciters fired at higher frequencies (Fig. 9B), although these units did not fire during ventilation (inset in Fig. 9B). Again, strong CI firing occurred just after arousal (Fig. 9B).

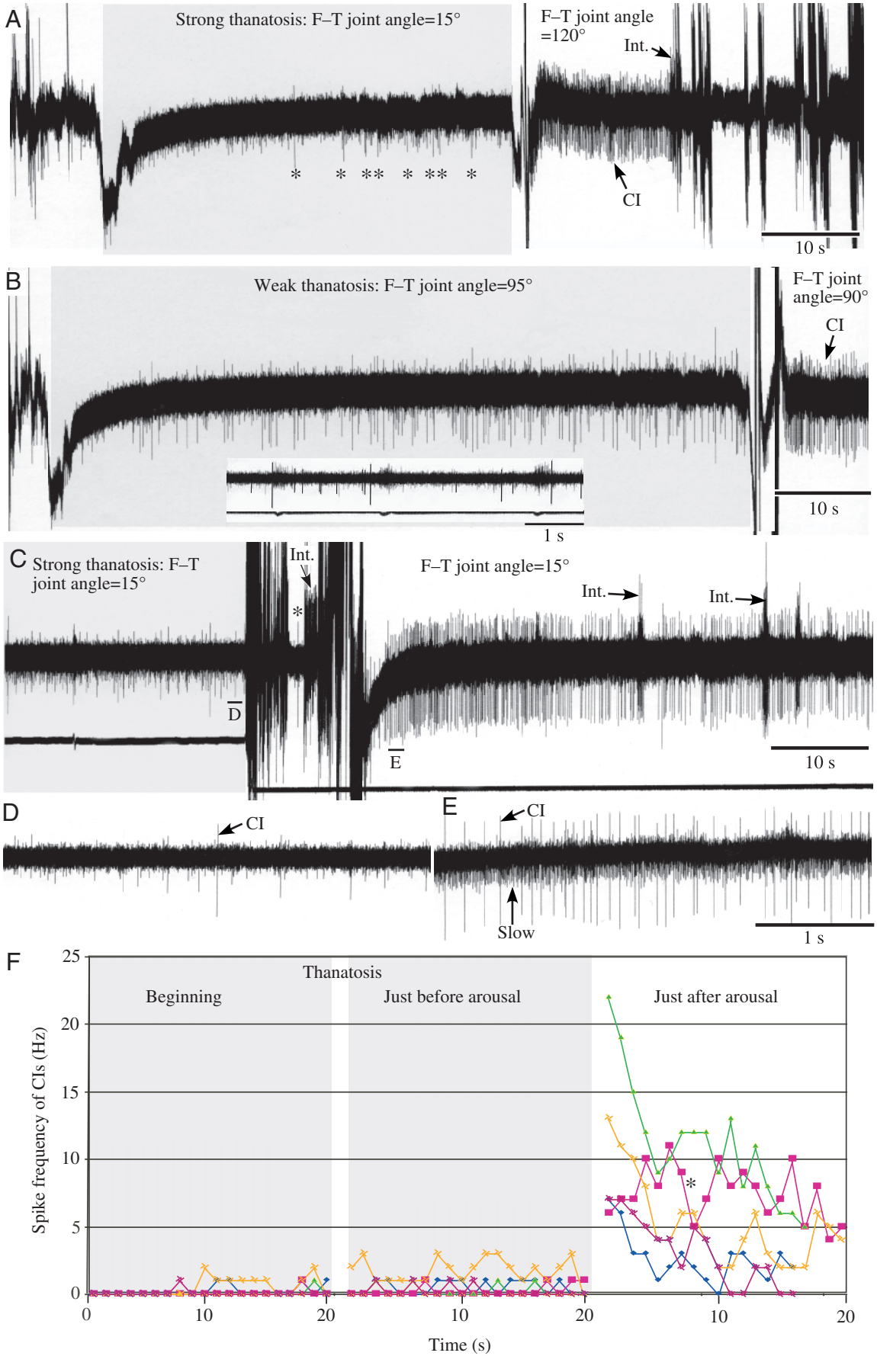
A
Strong thanatosis: F–T joint angle=15°
F–T joint angle
=120°
Int.
CI
10 s
B
Weak thanatosis: F–T joint angle=95°
F–T joint
angle=90°
CI
10 s
1 s
C
Strong thanatosis: F–T
joint angle=15°
Int.
F–T joint angle=15°
Int.
Int.
D
E
10 s
D
CI
E
CI
Slow
1 s
F
Thanatosis
Beginning
Just before arousal
Just after arousal
Spike frequency of CIs (Hz)
25
20
15
10
5
0
0
10
20
10
20
10
20
Time (s)

Fig. 9. Activity of common inhibitory motor neurones (CIs) before, during and after thanatosis. The maintenance phase of thanatosis is shaded by grey. (A) Strong thanatosis in which the recorded F–T joint was maintained at 15°. Activity of CIs were strongly suppressed especially in the beginning of thanatosis, then increased gradually towards arousal. Only eight spikes of CIs were identified in 42 s of the maintenance phase of thanatosis. The strong recruitment of CIs occurred immediately after arousal despite lack of motion of the recorded F–T joint at 120° (note that large exciters are inactive). The increased activity suddenly waned once the cricket started walking with recruitment of intermediate exciters (Int.). Large truncated spikes are cross-talk from large motor neurones. (B) Weak thanatosis in which the recorded F–T joint was maintained at 95°. CI started firing soon after induction of thanatosis and occurred frequently during thanatosis. Nevertheless, strong recruitment of CIs occurred immediately after arousal when the F–T joint was maintained at 90°. Note that CIs fire in non-ventilatory phases but the smallest slow exciter is activated during ventilatory phases (inset). (C) Arousal from strong thanatosis. Intermediate exciter was recruited with some cross-talk from large motor neurones. Note that a brief pause (asterisk) occurred when the cricket was struggling to right itself. Large spikes were truncated. (D,E) Time-stretched activity of labelled bars indicated in C. Note that strong recruitment also occurred in the slow exciters (causing apparent increase in baseline thickness) although the F–T joint was maintained same angle (15°) as in thanatosis. (F) Peri-stimulus time plot of CI activity in the beginning, just before arousal, and just after arousal. Five trials of thanatosis derived from three individuals are plotted. One trial (asterisk) indicates thanatosis induced in normal dorsal-up posture.

The distinct pattern of CI discharge was not affected by joint position, duration of the CI discharge nor by position in which the cricket was placed. For example, when the F–T joint angle was maintained at 120° (Fig. 9A), 90° (Fig. 9B) and 15° (Fig. 9C) immediately after arousal, CI discharge is similar in each case. A peri-stimulus time plot of CI activity in five trials of thanatosis (derived from three crickets) consistently showed that the CI activity was almost completely suppressed at the beginning of thanatosis but that the suppression declined gradually with the onset of ventilatory movements (Fig. 9D,F) and then strong recruitment of the CIs occurred on arousal (Fig. 9E,F). The CI discharge varied from 20–70 s, but still the characteristic high onset frequency adapted to a plateau level in each case (Fig. 9F). The pattern was unaffected by placing the cricket ventral or dorsal side up (asterisk, Fig. 9F).

As an intriguing note, just after attempting to right on arousal, the cricket paused suddenly in the ventral-side-up position for 2.5 s on the wooden bar. During this pause only one CI spike was observed (asterisk, Fig. 9C).

Discussion

Recording from small axons of slow exciters and CIs in the flexor tibiae muscle of unrestrained animals has been considered difficult (Wolf, 1990; Nishino et al., 1999). Coiling a fine wire directly onto the accessory flexor nerve was a break-through, allowing high-gain recording from both types of units simultaneously in a minimally dissected animal. This technique offers a clear advantage when elucidating neural mechanisms of static postural control, which largely includes the activity of small motor neurones.

General overview of the motor output defining thanatosis in the cricket

The main finding of this study is that motor output during thanatosis in the cricket is characterized by the decreased tonic activity in slow excitatory- and common inhibitory motor neurones to the flexor muscle. Thanatosis does not depend on the absence of motor output, nor is there any evidence for a catch-like mechanism in the muscle. Rather, muscle tonus (created by the weak discharge of the slow exciters) is maintained by suppression of CI activity that would otherwise relax the slow muscle fibres (Usherwood and Grundfest, 1965). These characteristics are generally in good agreement with those derived for other insects during thanatosis. In stick insects, only slow units were active while most of the other units were inactive (Godden, 1972). In the locust, there was little or no motor activity in any of the hindleg muscles (Faisal and Matheson, 2001).

The action of octopamine (released from DUM neurones) on the skeletal muscle has been investigated in several orthopteran insects. In locusts, octopamine reduces the amount of catch-like tension displayed by the extensor tibiae muscle (Evans and Siegler, 1977). By contrast, in wetas, infusion of octopamine (released from DUM neurones) into the slow extensor tibiae muscle and the subsequent stimulation of the slow extensor motor neurone led to catch-like tension (Hoyle and Field, 1983b). Similar effects were reported in the flexor tibiae muscle (Field, 2001). Although further investigation is clearly necessary, effects of octopamine appeared to be small for maintenance of the muscle contraction in crickets, as ablation of the distal flexor nerve that contains the DUM axons did not affect the flexed-leg posture during thanatosis (Table 1).

The most striking feature distinguishing thanatosis from other immobile states is ‘active suppression (presumably central inhibition)’ of the CIs, which was inferred from their marked excitation just after arousal despite the motionless of the leg (Fig. 9). As the CIs act by reducing residual tension in slow muscle fibres and speeding up ongoing leg movements (Usherwood and Grundfest, 1965; Burns and Usherwood, 1979; Wolf, 1990), tonic inhibition of the CIs during thanatosis promotes muscle rigidity and the maintenance of a particular stable posture. The post-arousal excitation (possibly post-inhibitory rebound) of the CIs reduces the muscle rigidity in preparation for locomotion. It is interesting that this period of the marked activation of CIs exactly coincides with that in which explosive escape-running occurs when the cricket is disturbed (Nishino and Sakai, 1996).

The motor effects of catalepsy involve muscles of all movable joints and segments (Nishino and Sakai, 1996), most of which are known to receive innervation from CIs (all six legs, Burrows, 1973; Hale and Burrows, 1985; Watson et al., 1985; Schmäh and Wolf, 2003; the antennae, Honegger et al.,

1990; body wall muscles, Yang and Burrows, 1983; Schmäh and Wolf, 2003). The inhibition of CIs is therefore very likely to occur commonly in posture-controlling muscles during thanatosis. CIs that are not associated with clusters of excitatory motor neurones (Fig. 5C,D) are now regarded as being clonally more closely related to inhibitory interneurones than to excitatory motor neurones (Wolf and Lang, 1994). This may underlie the anatomical observation that single CIs innervate functionally different muscles and relax them synchronously. It is known that even different CIs (e.g. CI1 innervates the extensor tibiae muscle while CI2 and CI3 innervate the flexor tibiae muscle) show synchronous activity because they receive many synaptic inputs in common, both in imposed movements (Hale and Burrows, 1985; Schmidt and Rathmayer, 1993) and in active walking movements (Wolf, 1990). These features have been difficult to understand in the control of active movements because relaxation should occur out of phase between antagonistic muscles (Hale and Burrows, 1985). However, the principle is potentially advantageous for the control of static posture, because the rigidity of all movable joints could be controlled at the same time by the activity of a relatively small number of CIs. Synchronizing the inhibition of CIs is especially important for survival because even small movements occurring in part of the body can elicit the attention of potential predators such as praying mantises (Yamawaki, 1998).

During thanatosis, fast and intermediate exciters were almost completely inactive while slow exciters were continuously active. This was also the case for the quiescent state. However, detailed observations indicated that exciters were held under weak inhibition during thanatosis. For example, fast units recruited in the 'leg flexion response (interganglionic response)' caused by pressing the prothorax on induction of thanatosis were not recruited when the same stimuli were applied during thanatosis (Nishino et al., 1999). The discharge of intermediate exciters that occurred during ventilatory phases in the quiescent state (Figs 4C, 6C) was suppressed during thanatosis (Fig. 4D, inset in Fig. 9B). A similar inhibition appeared to occur for the slow exciters because they discharged at a somewhat lower frequency in thanatosis than in the quiescent state (Fig. 8) and the enhanced activity often occurred just after arousal (Fig. 9E). It is known that motor neurones to the same muscle usually have a high proportion of postsynaptic potentials in common (Burrows and Horridge, 1974). This suppression of all exciter types may be attributed to the inhibition of premotor elements that excite the entire motor neuronal pool, as speculated by Godden (1972).

One might then ask why are only slow exciters continuously activated during thanatosis? Activation of the slow exciters occurs largely through persistence of the postural resistance reflex (Field and Coles, 1994) mediated by the femoral chordotonal organ (FCO), which contains position-sensitive sensory neurones as well as velocity- and acceleration-sensitive neurones (Burns, 1974; Matheson, 1990, 1992). This was revealed by an ablation study in which removal of sensory cells in the ventral part of the FCO scoloparia led to a severe loss of flexor muscle tonus in the respective leg during thanatosis (Nishino et al., 1999). Similar effects from inactivation of the FCO have been found in standing locusts (Usherwood et al., 1968).

I propose that the 'muscle plasticity (catalepsy)' typical of thanatosis depends on a weak discharge of the slow exciters producing a minimal force sufficient only to maintain particular tibial positions. More intensive recruitment of the slow exciters would produce a slow flexion movement instead of catalepsy (Hoyle and Burrows, 1973; Hoyle, 1980). In order to sustain a stable flexed-leg posture, despite the weakness of the muscle contraction, the accessory flexor muscle acts to mechanically stabilize the F–T joint synergistically with the inhibition of CI activity, as discussed below.

Functional roles of the main- and accessory flexor muscles in the cricket

Behaviourally, locusts do not walk quickly and escape by means of a powerful jump, whereas crickets show a less powerful jump but can run quickly (Tauber and Camhi, 1995). Underlying these behavioural differences, there are certain morphological and physiological differences in the flexor muscles of these two species. Most prominently, the locust main flexor muscle has nine exciters, each of which exhibits a unique and complex innervation to a restricted number of muscle fibre bundles (Phillips, 1980; Sasaki and Burrows, 1998); in contrast, the cricket has double the number of flexor exciters (about 18) but each innervates either the proximal-, middle or distal-muscle compartment with a simple, almost non-overlapping pattern (Fig. 3). Progressing distally, these muscle compartments insert into more distal points on the flexor apodeme/cushion complex at increasing angles and change from slow to predominantly fast innervation (Nishino, 2003). As a result, recruitment of the distal muscle compartment accelerates tibial flexion dramatically by the synergistic action of its increased effective leverage and rapid contraction. These properties must underlie the agility of the cricket, enabling the tibia to move quickly to the full flexion in running, jumping, kicking, and in the 'leg flexion response' on induction of thanatosis (Nishino et al., 1999). The cricket exhibits continuous acceleration of leg movements when a pulse of air is given during slow walking (Gras et al., 1994). Jumping and kicking readily occur, even during locomotion (Tauber and Camhi, 1995; Hustert and Gnatzy, 1995).

A unique morphology and neural innervation also occur in the accessory flexor muscle. Compared with locusts, the cricket accessory flexor muscle is well-developed (Fig. 2) and is functionally separated from the distal muscle compartment of the main flexor muscle due to the non-overlapping innervation of motor neurones (Fig. 5C). The origin of the accessory flexor muscle lies on the dorsal side of the F–T joint, while the origins of the main flexor muscle bundles lie on the same side (ventral) as the apodeme (Figs 2B, 5A). Because the accessory flexor muscle inserts onto the apodeme with such a large leverage angle, it pulls the cushion dorsally and obliquely to the normal proximal–distal path of action of the main flexor muscle. This

off-axis, oblique pull of the accessory flexor muscle has the effect of closing the joint with increasingly greater leverage as the F–T angle approaches zero (Hustert and Gnatzy, 1995; Nishino, 2003). Hence, due to this mechanical advantage (Heitler, 1974; Hustert and Gnatzy, 1995), the accessory flexor muscle effectively holds the F–T joint at 0° F–T joint angle during thanatosis. This was clearly demonstrated by the ablation study in which cutting the accessory flexor nerve resulted in greater deficiency in maintenance of the tibial flexion than in ablation of the proximal flexor nerve (Table 1), although both nerves have almost identical sets of slow exciters and although the proximal flexor muscle was larger in volume than the accessory flexor muscle (Nishino, 2003).

Due to its oblique insertion onto the flexor apodeme, the accessory flexor muscle has the joint-stabilizing role at any angle between 0° and 100°, corresponding to the range in which the flexor tibiae muscle functions (Nishino et al., 1999), by hindering the axial movement of the apodeme. Even with no electrical activity, the contractile viscosity of the accessory flexor muscle stabilized the joint, because cutting the accessory flexor nerve gave a smaller deficiency than ablation of the muscle bundles, in the maintenance of the tibial flexion during thanatosis (Table 1). This function seemed to be enhanced with suppression of CI activity, which prevents relaxation of the muscle, resulting in stiffening the joint. Indeed, the CI suppression exactly coincided with assuming sudden-immobile posture such as 'standing phase' (termed by Gras and Hörner, 1992) that occurs in escape trials (Fig. 9C), or 'thanatosis' that occurs immediately after forcible restraint of leg movements (Nishino et al., 1999).

In other orthopteran insects, the development of the accessory muscle (or comparable muscle bundles) seems to also relate to the joint stabilization function. For example, the stick insect *Cuniculina impigra* has a larger percentage of morphologically distinct bundles of slow fibres in the distal portion of the extensor tibiae muscle compared with the locust *Locusta migratoria*, leading to a stiffening of the leg joint to assume effective catalepsy (Bässler et al., 1996; Bässler and Stein, 1996). A particular tibial position of the metathoracic leg, assumed through avoidance conditioning, is maintained stably without any movements in the weta *Hemideina femorata* (Hoyle and Field, 1983a), which has a well-developed accessory flexor muscle as in crickets, but is 'fidgeted' in the locust *Scistocerca gregaria* (Hoyle, 1980), which has a small accessory flexor muscle (Matheson and Field, 2000).

CI activity may reflect arousal level

The present study enables the physiological characteristics of CIs to be defined more exactly. CI2 and CI3 in the cricket have similar physiological characteristics to those of the locust. For example, they had much lower tonic firing frequencies than the slow units (Burrows and Horridge, 1974). The two CIs (CI2 and CI3) showed very similar activity patterns that were often characterized by synchronous excitation (Figs 6, 7; Hale and Burrows, 1985; Wolf, 1990; Schmidt and Rathmayer, 1993). One recording showed an apparent difference in threshold between the CIs, with the smaller unit showing a higher background discharge and a higher tonic discharge to mechanical stimuli than the larger unit (Fig. 7).

However, in a strict comparison, background activity of CIs during quiescent state was different between these two species; whereas activity of the two CIs in minimally restrained locusts dropped 0 Hz (Wolf, 1990), that of minimally restrained crickets sustained tonically at ca. 0.5–2 Hz (Fig. 9). This difference may reflect the fact that the cricket tends to walk more actively than the locust, so relaxation of the slow muscle fibres due to activation of CIs may be important for starting locomotion smoothly. In the locust, the membrane potentials of CIs are maintained at a high level, even in the stance phase (static phase) of active walking (Wolf, 1990). Similarly, the much higher background activity of CIs (5–10 Hz, Figs 6, 7) detected in restrained crickets, compared to that in minimally restrained crickets, may reflect a high level of arousal driving an escape tendency.

These observations indicate that CI activity somehow increases with progressive arousal as noted in locusts (Wolf, 1990). The intensity and duration of the increased excitation were proportional to the strength of mechanical stimuli required for arousal (Fig. 7). Similar CI activation due to mechanical stimuli to sensory hairs on body appendages has been reported in cockroaches (Fourtner and Drewes, 1977) and in locusts (Runion and Usherwood, 1968; Hale and Burrows, 1985; Wolf, 1990; Schmidt and Rathmayer, 1993).

In contrast, strong inhibition of the CIs occurred during thanatosis, particularly in the beginning of the maintenance phase. The deeper in thanatosis, the more inhibition occurred in CIs (Fig. 9). Towards arousal, inhibition gradually declined (Fig. 9F). The arousal was characterized by a long-lasting excitation of the CIs (Fig. 9). Since this state was similar to CI activation caused by very strong mechanical stimuli such as pinching of the tarsus (Fig. 7D), one might speculate that self-stimulation involved with righting causes a tonic increment in CI activity. However, this is less likely for the following reasons. Firstly, the same excitation occurred in any other cases even without a righting response (Fig. 9F). Secondly, such a long-lasting excitation never occurred when the cricket was rotated during quiescent state. Hence, in thanatosis the cricket appears to be held at a very low level of arousal. Interestingly, the cricket showed vigorous palpal and antennal oscillation just after arousal (Nishino and Sakai, 1996), indicating 'awakening' from a state of low arousal.

Investigation of premotor elements using as CI inhibition as a reliable index of the thanatotic state is a future step to probe into the central function that operates this unique arousal mechanism.

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