

EFFECTS OF AFFERENCE SIGN REVERSAL ON MOTOR ACTIVITY IN WALKING STICK INSECTS (*CARAUSIUS MOROSUS*)

BY D. GRAHAM AND U. BÄSSLER

*Fachbereich Biologie,
Universität Kaiserslautern, D-6750 Kaiserslautern,
Federal Republic of Germany*

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SUMMARY

The apodeme of the femoral chordotonal organ of a middle leg can be moved from its normal position close to the extensor tendon and inserted into a clot cut in the flexor tendon. This inverts the output of the sense organ and produces a 'wrong' afference.

During walking on a pair of light wheels the operated leg either makes walking steps or is raised and extended in a 'salute' posture. The co-ordination is similar to that for an intact animal if the operated leg walks but changes to the middle leg amputee timing when the operated leg 'salutes'.

The transitions between saluting and the normal walking behaviour of the operated leg can be explained if it is assumed that the animal depends heavily upon the C.O. for determining the tibia position during both walking and the saluting behaviour.

Motor output to the levators and depressors of the femur and the protractors and retractors of the coxa shows bursting activity during the salute at the frequency of 3-4 Hz. The depressor bursts are also modulated at a frequency of ~ 1 Hz and produce strong regular depressions of the femur which are co-ordinated with the movements of the other legs.

The maintenance of regular depressor contractions during the salute shows that an important part of the motor output to the saluting leg (depressor activity) arises from an internal oscillation or rhythmic command which maintains its co-ordinated activity when the normal peripheral sensory input to the leg it is attempting to operate is absent. Retractor activity wanes during the salute suggesting that propulsion is much more dependent upon peripheral input than is the support musculature (depressors).

The creation of a 'wrong' afference can be used to map the importance of the operated organ in different sub-units of behaviour.

INTRODUCTION

Since the earliest observations of walking behaviour in insects numerous attempts have been made to disturb or, more usually, to destroy selectively, the sensory input of one leg. The purpose of such experiments is two-fold: to detect changes in the

timing or trajectory of the operated leg, which might be ascribed to the absence of feed-back from specific sense organs, and to search for changes in the co-ordination with other legs that might be directly associated with a particular sensory receptor.

The ablation of single sense organs has produced no significant changes in experimental studies of co-ordination and Wendler (1971) has shown that it is possible for several receptors to contribute to a perception system. This redundancy of detectors makes simple ablation an inadequate technique, as the animal may simply make use of other receptor systems. As an alternative to ablation, Bässler (1967) has shown that a surgically produced 'wrong' afference can produce dramatic changes in the behaviour of a leg. An afference is defined as 'wrong' if it signals a situation of the leg (position or stress) which is incorrect. The influence on the walking behaviour of the leg can be collectively summarized as follows: if a 'wrong' afference signals that a particular movement is incomplete the leg remains in that phase of movement and the transition to the next stage of movement is delayed, either briefly or indefinitely (Bässler, 1977).

In one of the above experiments a leg can be caused to break away from the normal walking rhythm at irregular intervals and remain in a raised and extended position. This 'saluting' behaviour is produced by surgical transfer of the receptor apodeme of the femoral chordotonal organ (C.O.) from its normal attachment point close to the extensor tibiae tendon to a notch cut into the flexor tendon. This is termed 'crossing' the receptor apodeme. This operation reverses the sign of the output from the C.O. so that extension of the tibia signals 'flexion' to the C.N.S. and vice versa.

The 'saluting' behaviour has been qualitatively described by Bässler (1967, 1977). This paper gives a more detailed quantitative analysis of the changes produced by the operation. The motor output to several muscles of the operated leg is examined in an attempt to understand the behaviour of the leg in the salute, and a precise description of the changes in co-ordination is given for the walking legs when the operated leg is saluting and when it is walking.

METHOD

Forty adult female *Carausius morosus* were operated upon to cross the femoral chordotonal apodemes (Fig. 1). The operation was performed on the anterior side of the femur 2–3 mm proximal to the femur-tibia joint. A small flap was cut on three sides and bent back to expose the receptor apodeme and flexor tendon. The tendon was cleared of one or two muscle fibres, and a notch cut in the tendon edge pointing in the proximal direction. The receptor apodeme was cut and the proximal part pushed into the notch cut in the flexor tendon. The flap of cuticle was allowed to fall back into place thus sealing the opening.

All operations were performed on the femur of the right middle leg during CO₂ anaesthesia. The animals were left immobilized for several hours to minimize the separation of the apodeme from its new attachment point. The effectiveness of the operation was tested in thanatotic animals by moving the tibia of the operated

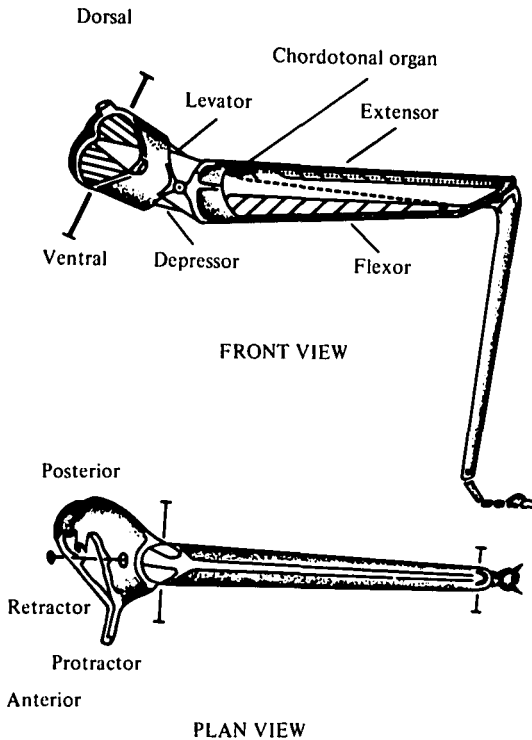


Fig. 1. Schematic drawing of the left leg of a stick insect showing the articulation and some of the musculature controlling leg movement. The chordotonal organ apodeme is shown in its normal position, . . . ; and after cutting and insertion into a slot on the flexor-tibiae tendon, ---.

leg in the direction of flexion or extension. An immediate strong movement in the same direction as the stimulus showed that the normal resistance reflex had been reversed (Bässler, 1967). The operation is usually very stable and the saluting behaviour produced by the operation does not alter for the remainder of the life of the animal (maximum 2 months).

Those animals in which the receptor apodeme pulls away from the tendon show no reflex of either sign but walk quite normally with no saluting behaviour. These animals provided a 'control' showing that attachment of the receptor apodeme to the flexor tendon with slight prestretch is essential for the observation of the saluting behaviour.

All the experimental data were recorded from animals walking on a pair of light Rohacell wheels 40 cm in diameter (moment of inertia 400 g cm²) mounted 3 cm apart on a common axle which was counter-balanced to give an upthrust against the animal of 50% of the body weight. For further information on the wheel system and a comparison of wheel walking and free walking behaviour, see Graham (1980a).

Only four animals showed poor 'reversal' or no resistance reflex after the operation.

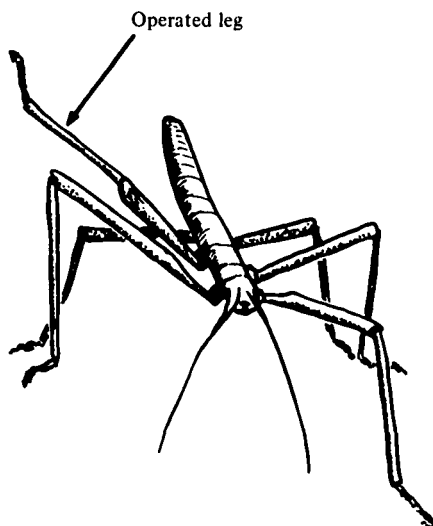


Fig. 2. Appearance of a free-walking animal during a 'salute'. The right middle leg has a crossed femoral chordotonal apodeme and the leg is shown extended and elevated at the forward extreme position of the coxa.

Of the remaining 36 animals, 13 were reluctant to walk on the flat, 15 walked poorly on the wheels with frequent struggling and walking backwards. Seven animals walked readily on the wheels for long periods, showed strong reversed reflexes and saluting movements and formed the data base for this paper. The animals were filmed for 60 s or less under a 100 W halogen lamp at a distance of 30 cm. The 30% success rate for wheel walking is typical of walking behaviour in free animals under these conditions of bright illumination.

The walks were filmed using a Beaulieu cine camera at 18 frames sec^{-1} . The position of the 'saluting' leg as a function of time and the co-ordination of the legs were evaluated from the film by means of a Hewlett Packard calculator and plotting table. Muscle recordings were made using insulated silver or copper wire and recorded on tape with a synchronizing signal from the film camera. Phase parameters were averaged using circular statistics (Batschelet, 1965).

RESULTS

Behaviour and motor output of the operated leg

A typical 'salute' posture for an operated animal is shown in Fig. 2. The animal stands or walks with the operated middle leg fully forward. The femur is strongly elevated and the tibia completely extended with the tarsus lifted. This 'salute' can appear in any leg if the receptor apodeme is crossed but all the results described here are for operations on the right middle leg. The hind legs show slightly more complicated behaviour and front legs show similar movements but are more difficult

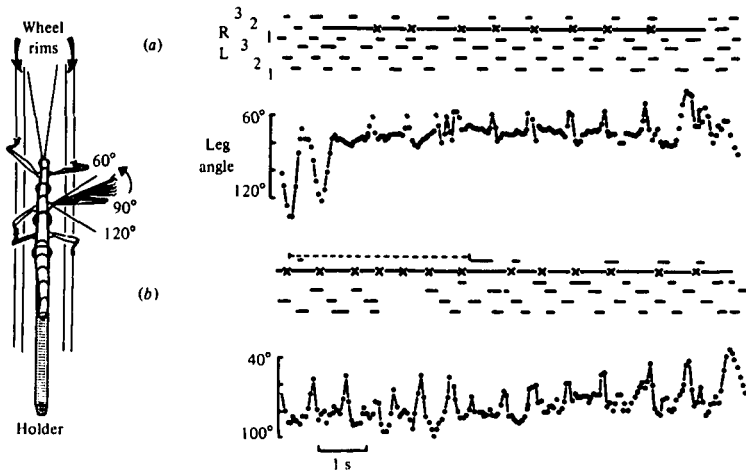


Fig. 3. The animal is shown attached to a rigid stick (holder) above two independent wheels. The right middle leg is held in the 'salute' position and the arrow denotes a forward and downward 'twitch'. (a) Step pattern of a 'saluting' animal. A black bar shows that the leg is not in contact with the wheel. An \times marks the beginning of a quiet period following a twitch or group of twitches. The position of the leg relative to the body is given below the step pattern and twitches are indicated by a brief forward and backward movement. (b) Step pattern from another salute in which the legs on the right stop walking for 4 s and the legs on the left momentarily increase their period from 800 ms to 1600 ms.

to operate upon (Bässler, 1977). The saluting position is seldom maintained continuously and in slowly walking animals the operated leg often steps normally for many cycles. If the animal is stimulated to walk faster then the operated leg may swing forward and instead of flexing, part way through the protraction stroke, the tibia continues to extend and the femur is rapidly elevated until the leg reaches the typical 'salute' configuration. The leg will remain in this position for at least one step period and typically remains elevated for 5–20 steps of the other legs.

During a 'salute' the tibia remains fully extended and the femur makes a regular sequence of sudden forward and backward movements or twitches with approximately the same period as the step frequency of the walking legs (Fig. 3). During such a twitch the coxa is already fully forward and moves very little relative to the thorax. The slight coxal movement that does occur is away from the body. The large apparent forward movement of the femur is produced by a depression of the femur relative to the coxa.

The motor output from four muscles of the leg is shown in Fig. 4. All records were made simultaneously with the film record. Examination of the walking part of the record shows typical walking steps of the operated leg. The motor output is similar to that found in an intact leg (Graham, 1980*b*). In retraction, depressors and retractors are active together while the levator and protractor are relatively inactive. Just before a protraction movement the levator activity begins. Protractors and depressors then fire briefly together the former to swing the coxa forward the latter opposing the levator activity. When the leg contacts the ground a brief burst

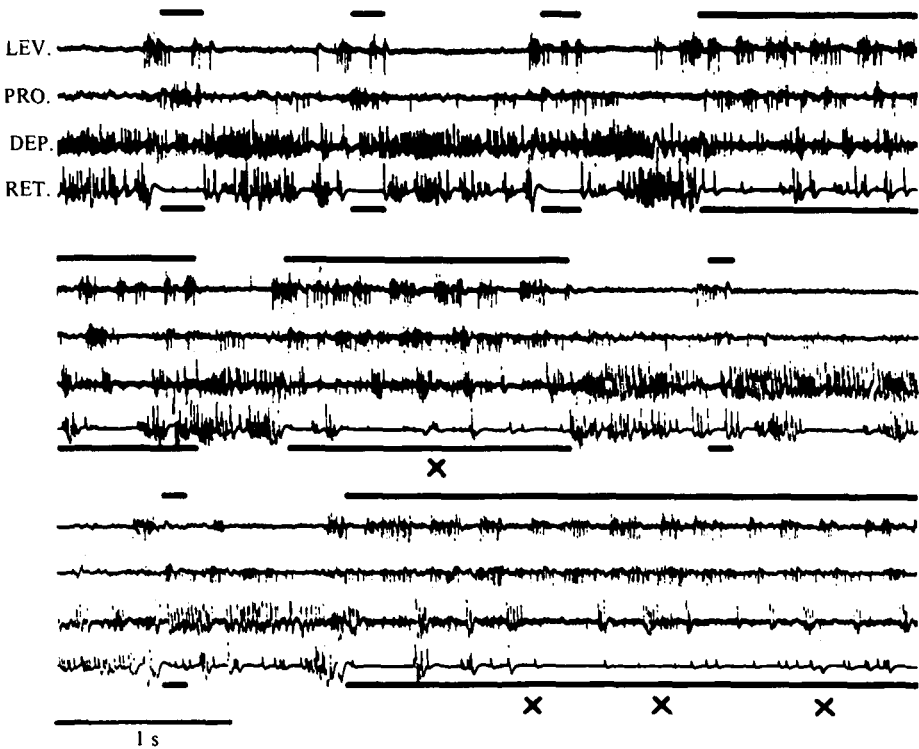


Fig. 4. Extracellular muscle activity from 4 muscles in the right middle leg in which the receptor apodeme is crossed (continuous record). The black bars are derived from a simultaneous film record and show that the leg is not in contact with the wheel. Short bars correspond to a normal protraction and the long bars represent a salute. The \times below two of the salutes corresponds to \times in Fig. 3 and denotes the beginning of a quiet period following a pronounced twitch observed in the film record.

from the levator appears which may be used to grapple the tarsal hooks to the walking surface.

During a 'salute' all these muscles are activated in short bursts at a much higher frequency than the ongoing step frequency of the other legs (in Fig. 3 ~ 1 Hz). The high frequency activity (~ 3 Hz) is higher than the maximum step frequency of adult stick insects but is in the frequency range of the seeking movements produced when the tarsi fail to make ground contact (3–4 Hz). The levator and protractor bursts are in approximate synchrony but in opposite phase with the depressor and retractor. The latter tend to fire synchronously at first but activity of the retractors rapidly decreases as the salute continues. The twitch movements show that the burst activity of the depressor muscle is modulated at the step frequency (~ 1 Hz) and the highest frequency bursts in the depressor produce an apparent forward movement of the leg, see Fig. 4. Notice that strong activity simultaneously in the retractors and depressors tends to cancel a forward twitch movement and a twitch is often not detected visually near the beginning of a salute. As retractor activity diminishes during the salute the forward movements become clearly visible.

The levator and protractor muscles possess the dominant mechanical advantage

and hold the coxa of the leg forward in the saluting position while brief intense bursts of the depressor produce a momentary twitch downward in the femur which is interpreted from above the animal as a forward movement of the leg. The twitch movements recorded during two examples of 26 long salutes (each > 5 steps) are shown in Fig. 3. The leg angle measured from the longitudinal axis in the horizontal projection shows that the twitches are large and well defined corresponding to an arc of more than 20° . In general the twitches are singlets but occasionally two or three occur very close together indicating the underlying higher frequency of depressor bursts. The mark (x) identifies the last of two consecutive angular displacements of greater than 10° and denotes the onset of a quiet period between twitches. This criterion was chosen because it is a reliable method of marking the end of either single or repetitive twitches.

The twitches themselves are also modulated in amplitude with a period of 3–5 s. Thus in both the salutes given here and in seven others the twitches increase slowly in amplitude, suddenly decrease, then progressively increase again. Usually the largest forward movement is associated with the end of a salute and this is often produced by the leg being depressed sufficiently to touch the wheel. As soon as the leg reaches the walk surface, or the tarsus is stimulated by a touch, the tibia promptly flexes and the leg is incorporated into the walking rhythm.

Co-ordination of leg movements during the salute

The timing of the beginning and end of salutes and the phase relations between legs are given in Fig. 5 and Table 1. They are summarized in the mean value step sequence at the top of the figure showing a typical salute. All salutes begin with a normal walking protraction of R₂. They occur spontaneously but can often be induced by touching the last segment of the abdomen. Following such a stimulus the walking speed increases and the saluting leg often lifts abruptly into the saluting position. The co-ordination of R₁ is most often unaffected by the beginning of the salute but in 30% of the salutes the first protraction of R₁ during the salute does not take place. The protraction is delayed for approximately one cycle of movement as shown in Fig. 5*a* and appears somewhat early in the next cycle of leg R₃ at a phase value of ~ 0.5 . This is the appropriate co-ordination for the remainder of the salute. During this step R₁ moves twice the normal distance during the retraction stroke relative to the body. This increased leg amplitude causes no overlap problem as the leg behind is lifted in a salute. Such absences in a metachronal sequence of protractions were not found in any of the walking sequences between salutes but such double length steps can occasionally appear in intact free walking animals (Graham, 1972).

During the salute the histograms show similar phase maxima to those of a middle leg amputee. The phase relations during the salute are slightly complicated by a wider distribution of phase values but essentially the co-ordination is the same. The widest phase distributions are those associated with leg R₃. This leg steps rather irregularly during the salute sometimes at a higher frequency than the other legs but more often at the same or a lower frequency. The lower frequency stepping is often caused by a failure to release the wheel. This particular behaviour greatly

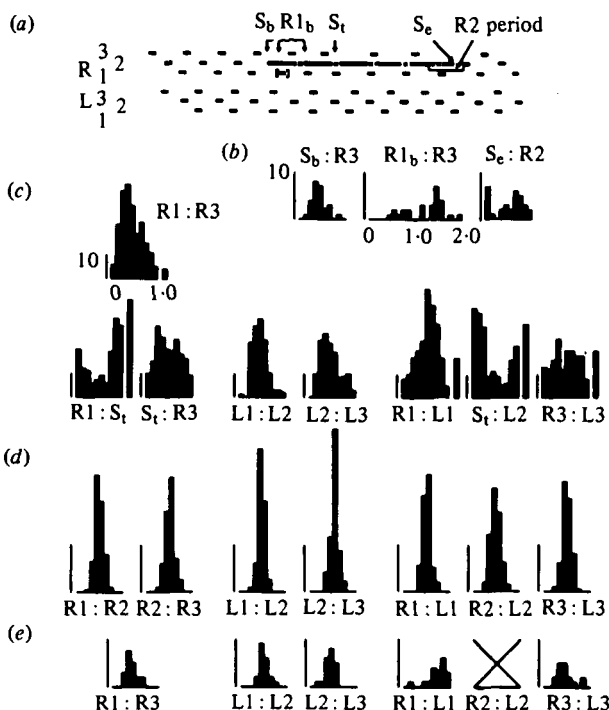


Fig. 5. (a) Step pattern of a representative salute derived from the mean values of the phase histograms given below. S_b marks the beginning of the salute. S_t corresponds to (\times) in Fig. 3 and S_e is the end of the salute. R_{1b} denotes the first protraction of R_1 following S_b . (b) Phase relations for the beginning and end of the salute. (c) Phase relations during the salute. Vertical scale is 10 in all histograms. $R_1:R_3$ denotes the phase of the start of a protraction in R_1 on the step of R_3 measured between the beginning of protractions. The range of phase is 0–1 unless marked otherwise and corresponds to 0–360°. A vertical column on the right of a phase histogram shows the number of reference cycles that contained no phase value. This indicates a higher average frequency in the reference leg for some walks. (d) Phase relations for an operated animal walking on the wheel system normally (no salute). (e) Phase relations for an adult amputee walking on the wheels.

perturbs the behaviour of the other legs producing a worse co-ordination than might otherwise be expected. This failure of leg R_3 to release the wheel prevents wheel rotation and often causes the front leg on that side to reach well forward. Under this condition the saluting leg may also reach forward, grasp the wheel and end the salute.

The salute can end in three different ways. In 30% of the salutes a large depression of the femur during a twitch causes the extended leg to touch the wheel. This occurs at a time co-ordinated with the other legs and a normal step co-ordination is resumed instantly. In 70% of the salutes the end point is distributed with an average end point one-third of the step period from the next protraction. The majority of these salute endings are caused by the failure of R_3 to release the wheel as already described but two salutes were terminated by a spontaneous flexion of the tibia causing contact with the wheel.

Fig. 5c also shows the co-ordination of twitches during the salute. While the co-ordination of the twitch (S_t) with R_3 is poor as would be expected from the

Table 1.

	R ₁ :R ₃	R ₁ :R ₂	R ₂ :R ₃	L ₁ :L ₂	L ₁ :L ₃	R ₁ :L ₁	R ₂ *:L ₂	R ₃ :L ₃
Operated salute*	0.38, 0.35, 211	0.92, 0.40, 151	0.44, 0.17, 186	0.43, 0.63, 129	0.41, 0.41, 133	0.58, 0.39, 217	0.11, 0.43, 182	0.53, 0.10, 192
Operated walk	0.87, 0.78, 78	0.41, 0.87, 80	0.44, 0.80, 78	0.43, 0.87, 80	0.56, 0.84, 82	0.55, 0.74, 80	0.54, 0.80, 80	0.66, 0.70, 82
Unoperated walk	0.01, 0.78, 113	0.50, 0.84, 119	0.52, 0.81, 115	0.48, 0.89, 117	0.54, 0.87, 121	0.51, 0.85, 114	0.48, 0.78, 120	0.50, 0.84, 116
Amputee	0.41, 0.75, 19	—	—	0.50, 0.83, 20	0.43, 0.81, 21	0.79, 0.61, 19	—	0.43, 0.59, 17

All data is for animals walking on a wheel (see Graham, 1980a).

First value is the mean phase, the second is the concentration parameter, the third is the number of phase measurements.

* In the salute R₂ timing is not a protraction onset but corresponds to (x) the moment immediately following a strong twitch in the saluting leg. This is denoted by the symbol S_t in Fig. 5.

irregular behaviour of the hind leg, the phase relationship between (S_1) and R_1 L_2 shows that co-ordination along and across the body is phase locked with a precision similar to that found between the legs on the unoperated side.

It is not possible to compare the twitching found here with the small movements seen in the leg stumps of middle leg amputees as the latter movements are somewhat inconsistent and more difficult to detect. However, Wendler (1965) suggested that the stump moves forward in synchrony with the front leg on the amputated side and it would, therefore, be expected to lag slightly behind protraction of the middle leg on the unoperated side (Graham, 1977). Thus the \times marking the onset of a quiet period following one or more strong twitches has a co-ordination with the other legs similar to that shown by the protraction of the leg stump of an amputee.

Frequently no twitch is observed after the first step period of the salute but twitching is rhythmically maintained throughout the remainder of the salute. As seen in Fig. 3*b* this regular rhythm can even be maintained during a period of reduced step frequency of the legs on the other side and a cessation of stepping on the same side caused by the wheel catching on the hind leg tarsus (R_3). This temporary independence in the timing of the forward twitch (S_1) has also been observed in two other salutes and it contributes towards the scatter in the phase of R_1 on S_1 compared to R_1 on R_2 in intact walking.

Co-ordination of the legs between salutes

In animals walking slowly, very long sequences of almost normal walking occur. References to Fig. 5*d* and Table 1 show that the co-ordination along the body is significantly non-tripod at this slower walking speed on the right side, with phase values that are significantly less than the unoperated walk values. On the left side L_1 and L_3 protract simultaneously. Across the body the phase relations (R:L) for front, middle and hind legs are all greater than 0.5. A left turning tendency was present in the free walking animals used for the data of Graham (1972) which produces a significant asymmetry in the phase locked pattern. In the salute operated animals walking on the wheels all phase relations across the body show a right turning bias and the phase asymmetry in each leg pair is significant ($P < 0.01$). This right turning tendency is consistent with a reduction in the force developed on the operated side caused either by damage to the muscles moving the tibia during the operation or the reversal in output from the chordotonal organ (D. Graham, in preparation).

At intervals (approximately once in every 5–10 steps) the right hind leg lifted in protraction and appeared to make searching movements (extending and flexing the tibia) 1–2 cm lateral to the rim of the right wheel. The other legs generally reduced their speed of movement or came to a complete halt during these oscillations of the tibia of leg R_3 . The amplitude of the waving movement increased with each oscillation until the tibia flexed sufficiently to contact the wheel rim. Subsequent re-examination of free walking data on both intact and operated animals and intact animals on the wheel showed no examples of such behaviour.

In contrast, all the non-saluting walks of operated animals confirmed that in every operated animal examples of such movements of the right hind leg occurred

h varying frequency (upper limit 1 step in 5). No such behaviour appeared in left middle legs and therefore the behaviour appears to be directly related to the operation on the adjacent right middle leg. On two occasions the right hind leg flexed under the body but in both instances the outside of the tibia touched the wheel rim on the first extension and the femur was levated and extended permitting the tarsus to grasp the wheel in the normal manner. The implications of this unusual behaviour are examined in the discussion.

DISCUSSION

The operated leg may either move normally in a walking step or it may 'salute'. The 'salute' itself is a direct response to the afference sign reversal following the displacement of the attachment point of the chordotonal organ from the extensor to the flexor side of the joint. An important question relating to the effect of sign reversal is whether the CNS must respond to the incorrect afference or can ignore it and use other afferences in its place.

In the walking behaviour between salutes velocity measurements for each wheel during a step cycle show that the force developed by the operated leg R₂ is half that of the leg L₂ in the same animal. These measurements will be described in a separate paper. Thus reversing of the chordotonal output reduces the forces developed by the leg during the retraction and creates a right turning bias in the phase locked relationship between right and left leg pairs (see Table 1). In addition, the frequent attempts by the leg R₃ to search for support outside the wheel rim suggest that the hind leg expects to find support 1–2 cm lateral to the position of the wheel rim. This behaviour relates to the work of Cruse (1979) in which he has shown that hind legs try to place themselves just behind the position of the retracting middle leg tarsus. Therefore, the lateral seeking movements which result directly from the afference reversal operation on the middle leg are most probably attempts to place the hind leg tarsus close to where the CNS believes the middle leg to be. This implies that the hind leg determines the lateral position of the middle leg tarsus by monitoring the output from the middle leg femoral chordotonal organ. Thus when the middle leg femur-tibia angle is at some value around 90° (depending upon the precise placement of the operated apodeme) the chordotonal organ reports the position of the leg correctly. When the leg moves away from this null angle, more medial positions of the tarsus are reported as more lateral and vice versa. The higher incidence of seeking movements by R₃ lateral to the wheel rim suggests that the leg is most often held slightly flexed compared to the null position. The incorrect reporting of the position of leg R₂ to leg R₃ and the possible role of the chordotonal organ in influencing the retractor motor output show that the CNS is using information from this sense organ during the walking behaviour. It continues to do so for weeks after the operation.

Clearly the reduction in motor output and the salute itself are disadvantageous to the animal and represent a dangerous reduction of mobility. This should place a strong pressure on the animal to adapt or modify its behaviour to correct the situation. However, saluting continues indefinitely with no obvious reduction in frequency. It would seem therefore, that the input from this organ cannot be ignored

and no alternative afferent pathway is able to replace its influence on certain parts of the behaviour. A similar failure to learn a new response has also been shown for immobilized leg walking in first instar stick insects (Graham, 1977).

With this hypothesis it is possible to provide a tentative description of how the leg moves in and out of the salute configuration. During slower walking the middle leg femur-tibia angle is maintained at $\sim 90^\circ$ during both protraction and retraction. In this null position the femur-tibia angle is signalled correctly even in operated legs. As the speed of the animal is increased the forward movement of the protraction stroke is accompanied by a more marked extension of the tibia. During the extension the operated chordotonal organ signals a pronounced flexion of the tibia to the CNS causing further extension until the leg is fully extended.

Under the fully flexed condition, which the CNS incorrectly assumes for the leg, the appropriate response is a levation followed by motor output to the extensor tibia. This would place the tarsus in a position where ground contact might be expected, and an oscillating or seeking movement could be used to search for ground contact. The protractor muscle is maintained in contraction to keep the leg forward and ready for a new retraction stroke. Notice that many other receptors such as position or possibly stress receptors could provide information on the true position of the leg. Such information does not appear to be acted upon in this particular behavioural context.

During the salute, depressor and levator muscles alternate in activity while protractor and levator muscles fire synchronously at a burst frequency of 3–4 Hz. The leg probably does not make large sweeping movements because the chordotonal organ continuously signals that the tibia is still folded under the body parallel to the femur. Thus the dominant motor output is that of levation and protraction. The full elevation is further re-enforced by the mechanical advantage given to the levators by this extreme position. A similar mechanical advantage is conferred on the protractor muscle but in this case as the salute continues the retractor activity in each burst declines until only a few units are rhythmically active.

The bursting activity of the depressors continues undiminished at 3–4 Hz but is modulated at ~ 1 Hz by co-ordinated output from the CNS which is sufficient to cause appreciable depression of the leg. This activity builds up over several seconds to produce a maximum depression. If the maximum depression is sufficient then the leg makes contact with the wheel. Stimulation of contact hairs, or bending stresses in the leg, signal ground contact and the leg flexes and the tarsus is attached to the walk surface. This ground contact response overcomes the chordotonal organ dependent behaviour of the leg during the long maintained protraction phase if any part of the tibia or tarsus is touched either by depression of the leg as above or by experimental intervention.

If the leg is maintained close to the 90° null position during the following protraction stroke then normal steps will follow. If the angle is appreciably less than 90° the tibia may flex under the body but will immediately contact the wheel by full depression of the femur and a normal step will follow. If the 90° null position is exceeded during the next protraction then the leg will swing up into the salute for the reasons already given.

The direct flexion of the tibia and depression of the femur upon contact between

ny part of the leg and the wheel rim or simply a stick held by the experimenter strongly suggests a hierarchy of reactions dependent upon different sensory inputs. In this example the specific sensory input produced by touching the tarsus overcomes the walking protraction programme which perceives the position of the leg exclusively through the femoral chordotonal organ. Presumably this can only take place if the hypothetical ground contact reaction does not depend strongly on this stretch receptor but uses hair plates or other position detecting organs to take-over temporary control of the motor system and produce the appropriate movements necessary to place the leg in a position to support the body. Such a change in the sensory perception of the position of the saluting leg must also occur in response to an increase in the force required to move the wheel caused either by a failure of the hind leg to release the wheel or the experimenter slowing the wheel by increasing friction at the rim.

During the 'salute' behaviour there is a strong and rhythmic activity in the depressor muscles at ~ 3 Hz which is additionally modulated at the step frequency of the other legs. The former and possibly also the latter oscillation appear to be able to operate independently of the other legs, as shown in Fig. 3*b*. The faster rhythm lies in the frequency range normally associated with seeking movements while the slower rhythm is a component of walking activity and suggests that the leg is trying to support the body at intervals which usually have the same periodicity as the walking legs and are co-ordinated with them. This confirms that a significant part of the motor output to the saluting leg arises from an internal oscillation which is able to sustain co-ordinated depressor activity when all of the normal peripheral sensory input to the leg it drives is absent.

The decay in retractor activity during the salute suggests that this muscle depends more strongly on the cyclic peripheral input and it is convenient to separate these two functions. Thus support (depressor activity) appears to be relatively independent of the peripheral sensory input while propulsion (retractor activity) although it still shows a weak intrinsic component requires a normal peripheral sensory input if the motor output is to be adequately expressed.

In the salute it is clear that the animal is walking and that an intrinsic component is present in the depressor motor output despite the absence of normal sensory feedback. This result supports the hypothesis of a co-ordinated centrifugal output generated either endogenously, as suggested by Wendler (1965) and Pearson & Iles (1973), or perhaps, in this instance, by the co-ordinated movements of the other legs.

The salute behaviour provides a temporary absence of the normal walking sensory feedback and the experiment appears to lie between amputation (autotomy) and recent experiments in which the intact leg is immobilized by attaching it to the body (Graham, 1977). The new results show that it is not the total absence of sensory input from femur and tibia which accounts for the amputee gait as suggested by earlier authors, but the absence of the *appropriate* sensory feedback. The salute operation appears to be less traumatic for the animal than immobilizing the leg and a large measure of co-ordination between front and hind legs is present although both these experiments are disturbed by a failure of the hind leg to release the tarsus from the substrate. This appears to be a support interaction in which the hind leg

sometimes fails to release if the middle leg is not in a supporting position. This behaviour does not occur if the operated leg is walking normally.

Throughout the salute the muscles controlling the movement of the leg hold the femur in a forward and strongly elevated position. These muscles are not continuously active but fire in bursts with a frequency of ~ 3 Hz and alternate with the depressor activity already described. As there is no further retractor activity shortly after the beginning of the salute, the leg is continuously held in a protracted position. The repetitive activity of the protraction musculature suggests that the leg is continuously attempting to protract but is unable to satisfactorily complete the protraction stroke perhaps because no signal has been received by the CNS showing tarsal contact. As an indication that this is indeed the case touching the tarsus causes immediate, strong, depression and flexion of the leg which produces ground contact in the free walking animal, as already described by Bässler (1967). Thus the salute behaviour can be considered to be an incomplete and prolonged protraction stroke.

If this interpretation of the salute is correct then such a prolonged protraction might prevent the protraction of the adjacent ipsilateral legs. As shown in Fig. 5b 30% of the first protractions of leg R₁ at the commencement of the salute are appreciably delayed. In the salute situation where the protraction of R₂ remains incomplete the operation of R₁ then appears to become independent of this delaying influence. Similarly, during the salute there appears to be an influence on the leg R₃ causing it occasionally to fail to release contact with the wheel. This again can be interpreted as suppression or a delaying of the protraction of R₃ by the saluting leg. This behaviour suggests that inhibitory influences arising from the middle leg are directed both anteriorly and posteriorly in this experimental configuration. However, these peripheral influences are weak and irregular compared to those influences which hold the unoperated legs in the co-ordination appropriate for the middle leg amputee and generate the co-ordinated depressions of the operated leg.

The hypothesis that the animal cannot ignore a wrong afference during a particular behavioural reaction is of considerable interest. It provides a simple explanation of the origin, maintenance and termination of the saluting behaviour and suggests that afference modifications of the kind described here can be used to map the function of a particular sense organ in the various elements of movement that make up the complex behaviour patterns of insects.

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