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GAIN CONTROL IN THE FEMUR–TIBIA FEEDBACK SYSTEM OF THE STICK INSECT

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Summary

This paper presents a quantitative description of the variability and the adaptive properties of information processing in the femur–tibia feedback system of the stick insect. The gain of this proprioceptive feedback system is determined by external stimuli changing the behavioural state of the animal and by internal properties that make it dependent on different parameters of the stimulus programme, e.g. stimulus frequency and amplitude, repetition rate and resting pauses.

The gain of the feedback loop in the inactive animal was investigated under open-loop conditions by applying mechanical sine-wave stimuli to the femoral chordotonal organ (fCO). The resistance movement of the tibia caused by these stimuli was measured with a new optoelectronic device.

A large increase or decrease in gain (up to a factor of 50) can be induced by stimulation, but also occurs spontaneously. The system shows habituation and sensitization. The initial gain can be decreased by repetitive sine-wave stimulation of the fCO. Disturbance of the animal (e.g. by tactile stimuli) increases the gain. The gain of the system decreases with increasing stimulus amplitude.

The described nonlinearities form a system which adjusts gain to a value that permits effective feedback and prevents instability. This was verified by closed-loop experiments.

Introduction

Behavioural and neurophysiological investigations show that many reflexes and neural pathways are active only under certain stimulus conditions or during certain states of behaviour of an animal. Also, quantitative changes in information processing, such as variation in the amplitude of the response to constant stimuli, have been mentioned in several studies on proprioceptive reflexes and feedback systems. Such quantitative changes in responsiveness are often due to different

stimulus conditions or behavioural situations (e.g. Forssberg *et al.* 1976; Cruse, 1981; Cruse and Pflüger, 1981; Cruse and Schmitz, 1983; Schmitz, 1985, 1986) and, therefore, might serve to adapt the system to the situation. However, even under identical stimulus conditions, large variations in response amplitude occur between individuals as well as in a single animal during an experiment (Graham and Wendler, 1981; Bässler, 1983; Kittmann, 1984; Schmitz, 1985, 1986). Very little is known about the reasons for these variations in information processing. They often exceed the differences found for the same pathway in different behavioural states or under different stimulus conditions. They have often been left uninterpreted or regarded as system inaccuracies rather than adaptive features, and have not been the subject of a systematic investigation until now.

In proprioceptive feedback systems there is a lack of quantitative data concerning such changes in the characteristics of the system. The variation in gain – the ratio between the output and the input of the system – is particularly important, as it can change the characteristics of the system considerably. Low gains result in ineffective feedback responses, whereas high gains can induce instability, e.g. oscillation of the system. Therefore, to maintain effective feedback, gain must be carefully controlled.

The femur–tibia (FT) feedback system of the stick insect is one of the best known proprioceptive feedback systems in arthropods. It has been investigated from the behavioural to the neuronal level (for a summary, see Bässler, 1983; Büschges, 1989). It stabilizes the FT joint against passive movements (Bässler, 1983) and is active during tibial movements (Cruse and Pflüger, 1981; Cruse and Schmitz, 1983; Cruse, 1985; Weiland and Koch, 1987).

For quantitative investigations, this system is almost ideal. Most animals become spontaneously active or change their state of behaviour during long-lasting quantitative experiments or as a reaction to applied proprioceptive stimuli, but this occurs rarely in the stick insect. Normally, the animal does not become active under daylight conditions and therefore the responses of its FT feedback system to the tested femoral chordotonal organ (fCO) stimuli are not obscured by active movements. It is easy to open the feedback loop and to stimulate the sense organ, the fCO, with an appropriate stimulus (Bässler, 1972a). The gain in the open-loop system can be calculated easily as the ratio of the output amplitude (tibial movement) to the input amplitude (fCO movement).

Quantitative investigations have shown the dependence of gain on different behavioural situations, in the standing and in the walking animal (Cruse and Schmitz, 1983; Cruse, 1985), and on different stimulus conditions, e.g. for different stimulus frequencies (Bässler, 1972a). However, these differences in gain are exceeded by the variations in the gain occurring under constant stimulus conditions and in constant behavioural states. Thus, the FT feedback system of the inactive animal showed a large variation in the response amplitudes for the following output parameters tested with constant stimuli: in the closed-loop system (i) the resisting force of the tibia against passive movements and (ii) the angular velocity of the return movement following a passive movement of the tibia

(Bässler, 1972*b*); in the open-loop system (i) the amplitude of FT angle (Bässler, 1972*a*; Bässler *et al.* 1974), (ii) the force produced by the tibia (Bässler, 1974) and (iii) the forces in flexor and extensor tibiae muscles caused by fCO stimuli (Storrer and Cruse, 1977). In spite of these intensive studies, little is known about the factors causing these response variations and influencing gain. Bässler (1974) and Storrer and Cruse (1977) assumed that a correlation between the state of behaviour of an animal and the gain in the feedback system explained this variability.

During my first experiments to find ways of reducing this variability in gain, I observed marked habituation and sensitization in the system. This made the results of a system analysis, e.g. the amplitude–frequency curve, strongly dependent on the stimulus programme, repetition rate and pause length and on random dishabituating influences.

In the following quantitative study, the variability in the characteristics of the system – in particular of the parameter 'gain' – was investigated and the following questions were addressed. (i) What gain values can occur and to what extent can they change within one individual? (ii) What factors induce these changes in the gain and what are their dynamic properties? (iii) Is it possible to increase or decrease the value of the gain by defined external stimuli under experimental conditions (an important prerequisite for a further investigation of gain control)?

Materials and methods

Adult female stick insects, *Carausius morosus* Br., were taken from a culture at the University of Kaiserslautern. All experiments were performed under daylight conditions at room temperature (20–22°C).

Preparation

The animal was mounted on a piece of foam rubber with its body axis vertical (Fig. 1). The femur of the left middle leg was stretched out perpendicular to the body axis, so that the tibia was free to move in the horizontal plane. The axis of the FT joint was adjusted to lie 1 mm above the centre of an optical angle measurement device. The coxa, trochanter and femur were then fixed with dental cement (Scutan).

Extracellular activity of the extensor tibiae motoneurones was recorded from the extensor nerve (F2) with 50 μm steel wires (Pflüger, 1977). Closed-loop experiments were performed under these conditions. For open-loop experiments, the fCO was mechanically stimulated as described by Bässler (1976): a pen motor (Hellige HE19) with a pair of forceps connected to its axis was used to move the chordotonal apodeme, which was cut distally at the FT joint. Stimulus functions were generated by a function generator (Tektronix, FG 501).

Optical measurement of the femur–tibia angle

A new optoelectronic device was developed to allow high-resolution recording

of the FT angle without manipulation of the tibia, which might change the friction of the joint. A lamp mounted 50 cm above the animal projected the shadow of the tibia on a 180° arc ($r=10$ mm) 1 mm below the tibia (Fig. 1). The FT joint was placed above the centre of the arc and the femur was fixed at the 0° line. The arc

Lamp

Nerve recording

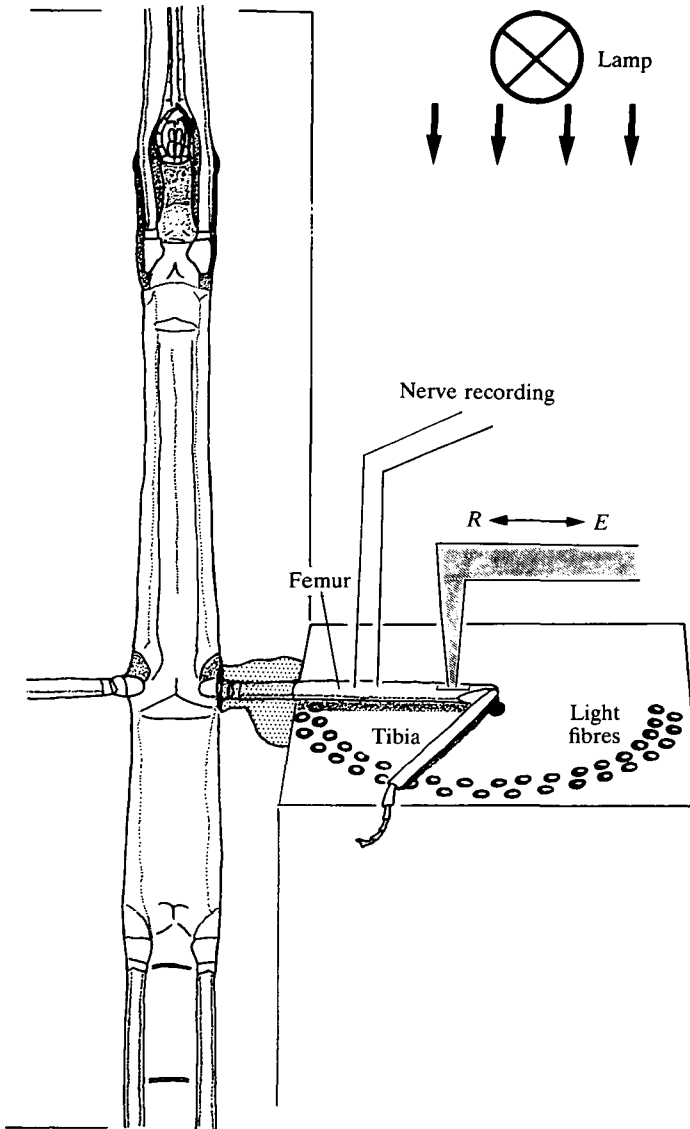$R \longleftrightarrow E$

Femur

Tibia

Light fibres

Fig. 1. Experimental arrangement. The animal is restrained in a vertical position. The left middle leg is fixed perpendicular to the body axis with dental cement. Two wires for extracellular nerve recordings (*nr*) and forceps to move the femoral chordotonal organ (fCO) (*R*, relaxation; *E*, elongation) are inserted into the femur of the leg. The tibia moves in the horizontal plane. A lamp projects the shadow of the tibia (*t*) onto the row of light fibres (*lf*).

was composed of light fibres installed every 5°. The diameter of each light fibre corresponded to 5°. Phototransistors connected to the light fibres detected the shadow of the tibia. An electronic device signalled the value of the FT angle through two output channels. The first channel indicated the location of the light fibre fully or partly occluded by the tibia, and showed the FT angle as a linear step trace, with every step corresponding to 5°. If more than one light fibre was occluded, the value of the light fibre with the highest angle (distal edge of the tibia) was used. The second channel registered the light intensity measured by this partly occluded light fibre and so gave a nonlinear high-resolution output of the FT angle within the actual 5° step. Individual calibrations for every light fibre allowed movements of less than 1° to be measured. This high-resolution channel only gives meaningful results for small movement amplitudes and, therefore, is only presented in Figs 2C and 7.

Tactile stimuli

Tactile stimuli were applied using a fine paintbrush. The intensity of the stimulus, its location and the frequency and force of the touch were varied. Acoustic monitoring of the activity of the extensor tibiae motoneurones helped to control the strength and effectiveness of the tactile stimuli. Small stimuli increased the activity of the slow extensor tibiae motoneurone only, whereas stronger stimuli also elicited single action potentials in the fast extensor tibiae motoneurone or even caused active movements with intense, irregular activity in both neurones.

During the quantitative measurements, the strength of the applied tactile stimulus was chosen so that one or only a few action potentials were elicited in the fast extensor tibiae neurone. Data from the electrophysiological recordings will be presented in a later paper.

Stimulus programmes

The elongation and relaxation movements applied to the fCO in the open-loop experiments were sine-wave functions. Stimulus amplitudes of 50–500 μm, corresponding to FT angles of 10–100° in the closed-loop system, and stimulus frequencies of 0.01–20 Hz were used.

The qualitative data presented in the first part of the Results are taken from experiments on 35 animals tested with variable stimulus programmes, under different stimulus conditions and in different behavioural states in order to describe the variability and the extreme values of the parameters of the system. In the second part, quantitative data about the habituation of the system to repetitive sine-wave stimuli with different stimulus frequencies and amplitudes were collected from five animals for every experiment. With standardized stimulus programmes, as much data as possible was gathered from each animal. Stimulation sequences of 50 continuous sine-wave cycles were chosen to habituate the system. After each stimulation sequence there was a pause of 4 min to allow the system to recover. Ten seconds before the next stimulation sequence began, a tactile stimulus of controlled intensity (see above) was applied to the abdomen of

the animal with a paintbrush to dishabituate the system. During the stimulus programme, stimulus amplitudes or frequencies were sequentially increased or decreased.

To determine the gain during the pauses between the stimulus sequences, short test stimuli of 1–3 sine-wave cycles were used. Owing to the long pauses and the low-frequency stimulus sequences, measurements took up to 8 h. At the end of the experiments, the animals were tested for normal physiological reactions. The ability to flex and extend the tibia fully during active movements was tested. Data recorded at the beginning and at the end of the experiments were compared.

Calculating the gain

In the closed-loop system of the middle leg, an FT angle of 20° causes a 100 μm movement of the fCO apodeme (Kittmann, 1984). The gain of the open-loop system was therefore calculated as the ratio of the output (the amplitude of an FT angle) to the input (the amplitude of the fCO movement). In calculating gain for different reaction amplitudes, the limitations of the method must be taken into account. The measurement of small amplitudes is limited by the resolution of the angle measurement device. For channel 1 it is 5°. For large reaction amplitudes, the correct value for the internal gain of the feedback system can only be calculated if the tibia does not reach one its the extreme positions during its movement. Otherwise the internal gain of the system might be larger than the calculated value. As minimum and maximum amplitudes were limited to 5° and 155°, it was possible to measure values of gain in the range 0.5–15 or 0.05–1.5 with the stimulus amplitudes of 50 or 500 μm, respectively. With the necessary calibrations and evaluations, the high-resolution channel allowed quantification of movement amplitudes to 1°. This was especially important for the calculation of the maximum factor of gain change.

Data acquisition and evaluation

The electrical signal of the pen motor (stimulus function), the two channels of the FT angle measurement device and nerve recordings were stored on a d.c. tape recorder (Racal Store-4). For further evaluations, recordings were registered with a pen recorder (Hellige He 18). The FT angle was evaluated from the 5° channel of the angle measurement device. Statistical analyses were carried out with the aid of a computer system (Apple 2+). Wilcoxon–Mann–Whitney U-tests or paired t-tests (Sachs, 1974) were used and values of probability (P) below 0.05 were taken as significant. Time and cycle constants of the exponential decrease or increase in gain were taken as the slopes of the regression lines, calculated from the data points of the logarithmic form of the exponential equations.

Results

For a better understanding of the numerous factors influencing the character-

istics of the system, the results are first described qualitatively. Quantitative data on certain aspects are then presented.

Qualitative description of the variability and adaptive features of gain
Reactions to tactile stimuli

Tarsi, mouthparts, antennae and cerci were most sensitive to tactile stimuli. Slight stimuli normally led to an increase in gain, causing the FT angle to change slightly. Strong tactile stimuli often elicited active searching or struggling leg movements which lasted for 2–30 s.

The sensitivity of the animals to tactile stimuli showed remarkable individual variation. A light touch with a few hairs of the paintbrush elicited active movements in some animals, but others reacted only to strong repetitive touches on the most sensitive parts of the body. Animals could also exhibit marked changes in sensitivity during an experiment. In most cases there was a gradual desensitization to tactile stimuli during the experiment. However, in several experiments sensitization was observed: although at the beginning of the experiment strong tactile stimuli were necessary to elicit active movements or a large increase in gain, after such a reaction had occurred, the animal was extremely sensitive to these stimuli for the following minutes and even faint stimuli immediately elicited activity or large changes in gain.

Increase in gain in response to tactile stimuli (sensitization)

For all fCO stimulus frequencies (0.01–2 Hz) and amplitudes (50–500 μm) tested, tactile stimuli to any part of the body increased the gain of the system (Fig. 2B, see quantitative data below). It could be increased during continuous fCO stimulation as well as during the pauses between sine-wave stimuli. The increase in gain was normally correlated with the strength of the tactile stimulus and desensitization in response to repetitive tactile stimulation could be observed. After a tactile stimulus there was only a small decrease in gain with time as long as no repetitive fCO stimuli were applied (see Fig. 3 and quantitative data below).

Decrease in gain caused by continuous fCO stimulation (habituation)

During continuous sine-wave stimulation, the amplitude of the FT angle decreased (Fig. 2A,B; see quantitative data below). This held for all tested stimulus frequencies and amplitudes. The gain was not only decreased for the frequency of the stimulus sequence at which it was habituated, but also for other frequencies (stimulus generalization, see quantitative data below). During the pause following repetitive stimulation, the gain increased spontaneously and reached about 90 % of its initial value (spontaneous recovery, see quantitative data below and Fig. 6). Changes in gain, both increases (caused by tactile stimuli) and decreases (caused by repetitive stimulation), were reversible. As demonstrated in Fig. 2B, gain can be increased and decreased several times during the same stimulus sequence.
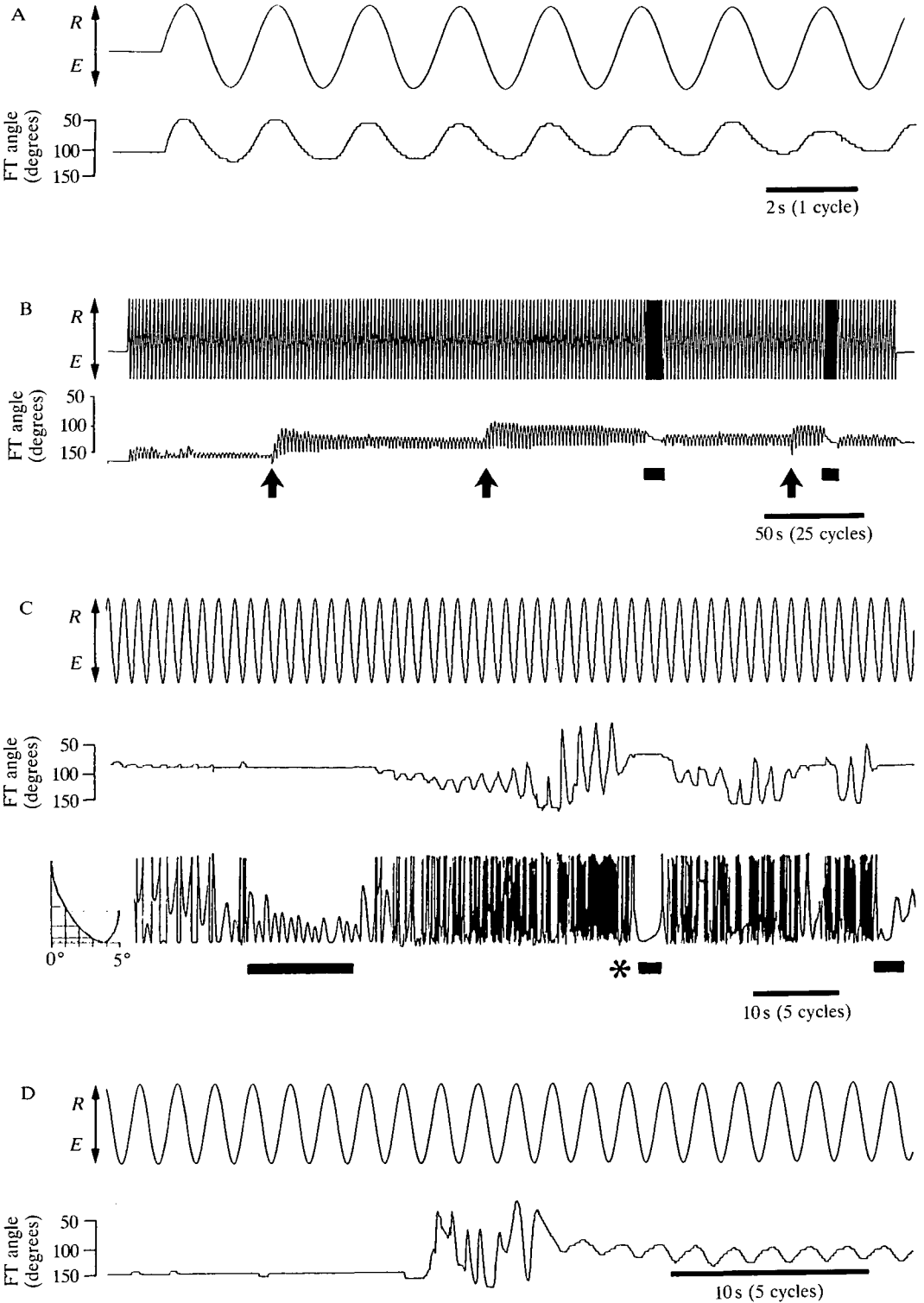
Fig. 2

Fig. 2. Resistance movement of the tibia in response to sinusoidal fCO stimuli. (A–D) First trace, fCO stimulus, position of the fCO apodeme; E, elongation; R, relaxation; 0.5 Hz, 300 μm. Second trace, femur–tibia (FT) angle (degrees), each step corresponds to 5°. Third trace, high-resolution channel (only in C). (A) Decrease in response amplitude (lower trace) during continuous sinusoidal stimulation. (B) Habituation by repetitive sinusoidal fCO stimulation and dishabituation by tactile stimuli (arrows). Bars mark the habituation of the system with an increased stimulus frequency (40 cycles, 5 Hz, 300 μm). (C) Large spontaneous changes of gain. The maximum amplitude of FT angle is 150° (second trace). During the sequences marked by bars, the amplitude is less than 5°. At the second bar (*), the response amplitude is below 0.5°, as no response can be detected in the high-resolution channel (third trace). As a calibration for this nonlinear channel, the output function of a linear 5° movement is indicated on the left. (D) Spontaneous active movements, followed by an increase in the gain.

Variation in gain

To illustrate the variation in gain, the minimum and maximum reaction amplitudes of all experiments were evaluated. For a stimulus amplitude of 300 μm in the frequency range from 0.01 to 0.5 Hz, for each tested frequency, maximum reaction amplitudes of 150° and minimum reaction amplitudes below 5° were found. This shows that the gain of the FT feedback system can change by more than a factor of 30. The value of this factor was limited by the resolution of the measurement of FT angle and by the physical limits of the joint movement. To document the changes in gain for individual animals, several extreme changes in gain, occurring close together during the experiments, were analysed. With the calibration of the high-resolution channel allowing quantification of changes in the FT angle to less than 1°, changes in gain of greater than 50-fold were detected in several animals. These changes could occur within a few seconds (Fig. 2C).

Spontaneous variation in gain without any visible active movements of the other legs occurred, although rarely, either during (Fig. 2C) or between fCO stimuli. In these cases, the d.c. value and amplitude of the FT angle could change quickly and independently. After an induced or spontaneous active movement, gain could change unpredictably, either increasing or decreasing (Fig. 2D).

Quantitative description of the characteristics of the system

Decrease in gain with time

The observation that gain can be increased quickly by tactile stimuli raises the question of how the gain changes with time after such a stimulus. Because the gain of the system cannot be measured without applying fCO stimuli, which habituate the system and decrease the gain, single sine-wave cycles (0.5 Hz, 300 μm) were used for this purpose. They were applied 10, 50, 100, 200 and 500 s after a tactile stimulus. Twelve experiments were performed with five animals. The value of the gain at $t = 10$ s was taken as 100 % (Fig. 3). Between the tenth and fiftieth second, a significant decrease in gain to about 90 % occurred. The gain then remained at approximately this value.
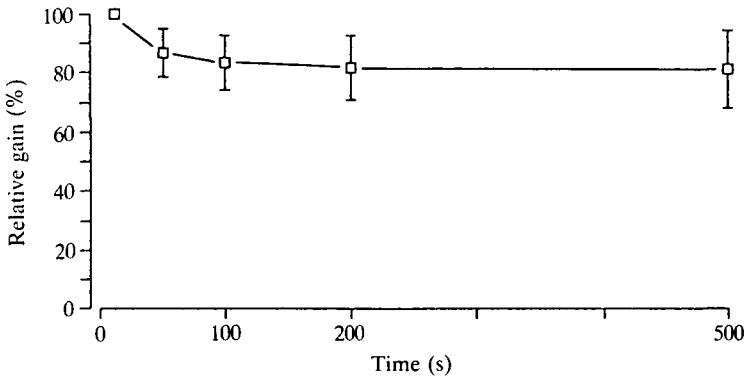
Fig. 3. Mean decrease in gain measured with single sine-wave cycles during the time following a tactile stimulus ($t=0$). The first value measured at $t=10$ s was taken as 100 %; $N=12$ from five animals; standard deviations are indicated.

Decrease in gain in response to repetitive stimulation (habituation)

Gain versus *stimulus amplitude.* In linear feedback systems, gain is independent of the input (stimulus) amplitude. As biological systems often show nonlinearities, the gain and its quantitative decrease in response to repetitive sine-wave stimuli were investigated for the stimulus amplitudes 50, 100, 300 and 500 μm at a constant stimulus frequency of 0.5 Hz. The amplitudes of the first, fifth, tenth, twentieth and fiftieth movements during sinusoidal stimulation of the fCO were evaluated. The experiments were carried out on five animals, and stimulus sequences for each amplitude were tested at least 12 times. The amplitude of the FT angle increased with the stimulus amplitude. For the first and for the fiftieth sine-wave cycles, the response amplitudes to 500 μm stimuli were significantly higher than those to 50 μm stimuli. In all experiments and for all stimulus amplitudes, a significant decrease in response was found from the first to the fiftieth cycle of a stimulation sequence (paired t-test, $P<0.01$). In many cases, a constant value was reached between the twentieth and fiftieth stimulus cycle. The decrease in amplitude with time can be fitted as an exponential function of the form:

$$A(t) = A(t0)e^{-t/T} + B, \qquad (1)$$

where t is the time from the beginning of the stimulus sequence (s), $A(t)$ is the amplitude of the FT angle at time t (degrees), $A(t0)$ is the amplitude of the FT angle at time zero (degrees), T is the time constant (s) and B is the constant amplitude of the FT angle (fiftieth cycle, degrees).

The time constant was calculated for every stimulation sequence. The extreme values, for all experiments, were 12 and 110 s. The mean values for the different stimulus amplitudes were between 18 and 28 s. They did not show any systematic dependence on the stimulus amplitude. The values of the gain calculated for the different stimulus amplitudes showed a previously unknown nonlinearity of the
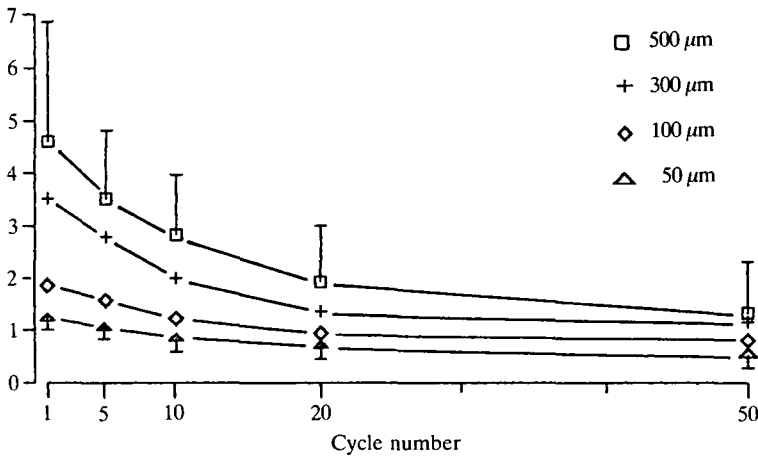
Fig. 4. Gain plotted against cycle number during a stimulation sequence (0.5 Hz) at different stimulus amplitudes. Mean values (N=12, five animals) with standard deviations are given for the stimulus amplitudes 50 and 500 μm.

feedback system, with the gain decreasing with increasing stimulus amplitude. This was valid for the mean values of the first, fifth, tenth, twentieth and fiftieth stimulus cycles (Fig. 4). The decrease in gain was significant for the first and the fiftieth cycles when comparing the values of the 50 and 500 μm stimulus amplitudes (*t*-test, $P<0.05$). An increase in stimulus amplitude by a factor of 10 (from 50 to 500 μm) resulted in a mean decrease in gain by a factor of 3.8 (first cycle) to 2.3 (fiftieth cycle).

Gain versus *stimulus frequency*. To investigate the mechanisms underlying gain changes, the time course of changes in the amplitude of the response to repetitive sine-wave stimulation was measured for stimulus frequencies from 0.01 to 5 Hz at a constant stimulus amplitude (300 μm). The stimulus programme corresponded to the one described above and was carried out on five animals. Owing to their long duration (50 cycles=5000 s), the stimulation sequences at 0.01 Hz were tested only five times, whereas all other frequencies were tested 16–18 times. For all stimulus frequencies, significant decreases in the gain from the first to the fiftieth stimulus cycle were found (Fig. 5). Depending on the stimulus frequency, the gain declined to 5–30 % (first cycle=100 %) during a stimulus sequence. The amplitude–frequency plot of the system changed not only its absolute values but also its shape during the decreases in gain, showing that the filter characteristics of the system change considerably. The time constants of the decrease in gain depended on the stimulus frequency (Table 1). These time constants can be converted into cycle constants that describe the decrease in response as an exponential function of the number of cycles applied during a stimulation sequence. The average values of cycle constants (Table 1) differ only by about a factor of 1.5 for a stimulus
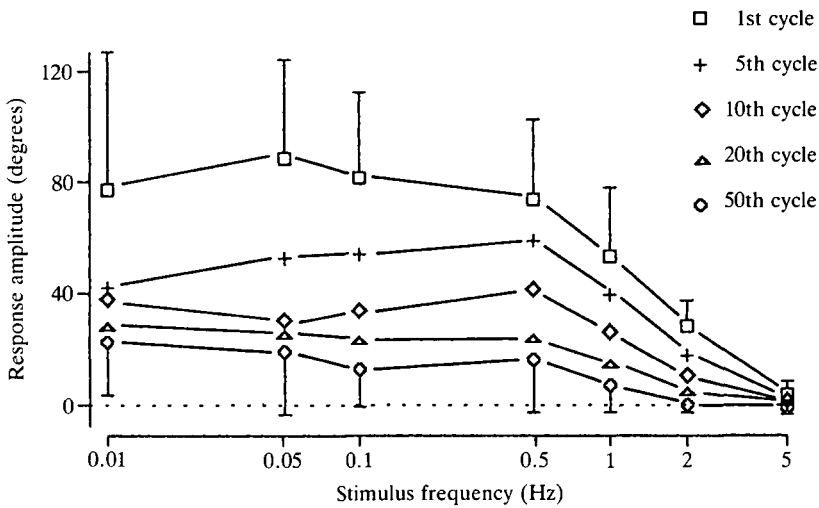
Fig. 5. Amplitude–frequency response curves from the mean values of the first, fifth, tenth, twentieth and fiftieth cycles of stimulus sequences. Standard deviations are given. For further details see text.

frequency range from 0.01–2 Hz and show no clear dependence on stimulus frequency:

$$A(c) = A(c1)\mathrm{e}^{-c/C} + B, \tag{2}$$

where c is the cycle number from the beginning of the stimulus sequence, $A(c)$ is the amplitude of the FT angle at cycle number n (degrees), $A(c1)$ is the amplitude of the FT angle at the first cycle (degrees), C is the cycle constant and B is the constant amplitude of the FT angle (fiftieth cycle, degrees).

Is the decrease in gain in response to repetitive sine-wave stimulation frequency-specific (stimulus generalization)?

     Repetitive sine-wave stimulation at a given stimulus frequency decreases the

Table 1. *Average values of time constants (T), and cycle constants (C) with their standard deviation (S.D.), describing the course of the decrease in gain during continuous sinusoidal stimulation*

	Stimulation frequency (Hz)						
	0.01	0.05	0.1	0.5	1	2	
T (s)	1390	186	124	28	11.8	6.2	(s)
C	13.9	9.3	12.4	14.0	11.8	12.3	
S.D.	4.0	3.6	5.0	5.4	5.1	4.0	

gain and, therefore, the question arises of whether this decrease is frequency-specific or whether the gain is decreased for the whole stimulus frequency range to which the system responds. This was tested with a stimulus programme in which gain was examined with three sine-wave cycles of 0.5 Hz before and after a stimulation sequence at another frequency (0.01, 0.1, 2, 10 Hz). The percentage decrease in gain between the first and fiftieth stimulus cycles and between the test cycles applied before and after the stimulus sequence were compared.

In all 32 experiments carried out on five animals, the decrease in gain caused by the stimulation sequence led to a similar decrease in gain in response to the test frequency. The percentage decrease in gain showed some variation between the frequency of the stimulation sequence and the test frequency (up to a factor of 2). Ten experiments gave similar results for test frequencies of 0.1 and 2 Hz and stimulation sequence frequencies between 0.01 and 2 Hz.

Spontaneous recovery of gain after habituation

During the pause following a decrease in gain caused by a stimulation sequence (0.5 Hz, 300 μm, 50 cycles), a spontaneous exponential increase in gain was measured. In 12 experiments on five animals, single sine-wave cycles (0.5 Hz, 300 μm) were applied 10, 50, 100, 200 and 500 s after the end of the stimulation sequence to investigate this spontaneous recovery of the gain. The amplitude of the first cycle of the stimulation sequence was taken as 100 %. The percentage increase in gain resembles an exponential function:

$$A(t) = A(0)(1 - e^{-t/T} + B, \qquad (3)$$

where t is the time from the beginning of the resting pause (s), $A(t)$ is the relative amplitude of FT angle at time t (%), $A(0)$ is the amplitude of the FT angle at the first cycle of the stimulus sequence (taken as 100 %), T is the time constant (s) and B is the relative amplitude of the FT angle at the fiftieth cycle of the stimulus sequence (%).

Following a rapid recovery in gain in the first 100 s to about 70 % of its value before habituation, the gain reached a mean value of more than 90 % at the end of a 500 s rest (Fig. 6). There is a strong correlation ($r=0.84$, $N=12$) between the value of the gain in the first cycle of the stimulus sequence and the value to which gain recovers after a pause of 500 s. Time constants for the recovery had a mean value of 99.5 s and showed marked variations (s.d. 97 s).

Instability and habituation in the closed-loop system

The results describe nonlinearities of the FT system that could prevent possible instability resulting from an increase in gain above a critical value. This could be accomplished by habituation and by the decrease in gain with increasing stimulus amplitude. Closed-loop experiments were performed to test whether these properties are used for this purpose.

The gain of the closed-loop FT feedback system was increased by tactile stimuli. This resulted in regular oscillations of 2–4 Hz, corresponding to the resonance
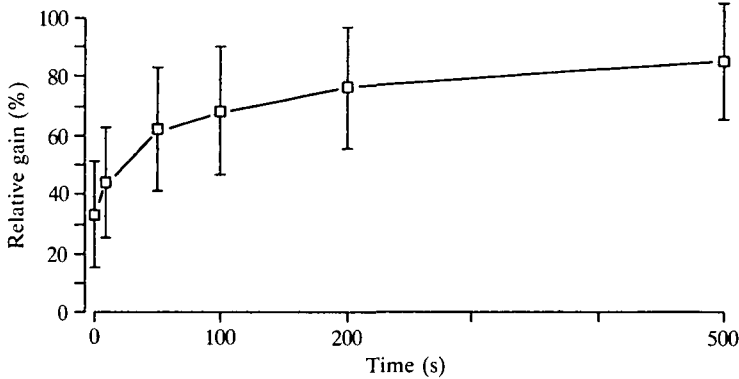
Fig. 6. Spontaneous recovery of gain after habituation to a stimulus sequence. Mean values ($N=12$, from five animals) with standard deviations are given. For further details see text.
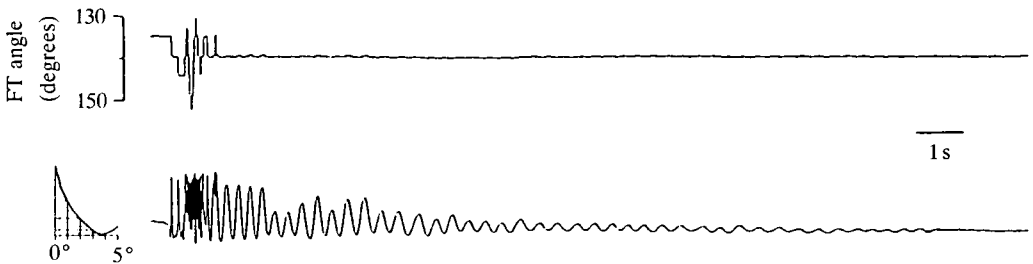

Fig. 7. Oscillation of the closed-loop femur–tibia (FT) feedback system caused by a tactile stimulus increasing the gain. Within a few seconds, the system re-establishes stability by decreasing gain as a result of habituation. Upper trace, FT angle, each step corresponds to 5°; lower trace, high-resolution channel. As a calibration for this nonlinear channel, the output function of a linear 5° movement is indicated on the left.

frequency of the feedback system. The oscillations had initial amplitudes of 25–5° which then decreased exponentially. During the next 5–20s, their amplitudes decreased below 0.5° and a stable tibial position was reached (Fig. 7). The decreases in gain had time constants between 1 and 3s, corresponding to cycle constants of about 3–9 cycles, values comparable to those found with habituation in the open-loop system. These results strongly indicate that habituation is one of the mechanisms that stabilize the FT feedback system.

Discussion

The FT feedback system in the resting animal exhibits variability in its response to constant stimuli. The gain can vary by up to a factor of 50 in a single animal,

which far exceeds the variation found for different behavioural states. This variability resembles that of the adaptive properties of the FT feedback system. It is not random, but is caused by external and internal inputs onto the gain control system. The gain is influenced by input to its own sense organ, the fCO, by stimuli applied to other sense organs, possibly changing the state of arousal of the animal, and by the internal dynamic characteristics of the system, e.g. the time course of recovery.

Some of these properties, summarized under the terms habituation and sensitization (for a review, see Thompson and Spencer, 1966), are well known for reflexes but their existence in proprioceptive feedback systems has not been demonstrated before. Unlike reflexes, where a stimulation of a sense organ elicits a reaction in the effector organ, feedback systems require a continuous circular flow of information in the feedback loop to control the regulated parameter. This demands accurate and quantitative information processing, otherwise instability or an ineffective feedback reaction could occur. Thus, in feedback systems habituation and sensitization serve additional functions in gain control and system stabilization (see below). Other characteristics of the system, such as the decrease in gain with increasing stimulus amplitude, have not previously been described for this proprioceptive feedback system.

Results on proprioceptive feedback systems of the other leg joints indicate that the characteristics of the system may also be present in these feedback systems. Thus, the forces resisting imposed limb movements from the femur–tibia, coxa–trochanter and subcoxal feedback system of the stick insect (Schmitz, 1985) show similar response characteristics and a comparable variation (standard deviation) in their response amplitudes. For the coxa–trochanter feedback loop, Schmitz (1986) found remarkable variations in the standing animal, even for the average values of force from different individuals (up to a factor of 3), whereas changes in gain due to different behavioural states – from standing to walking (Cruse and Schmitz, 1983; Schmitz, 1985) – only caused comparable changes in gain.

Habituation and sensitization in the femur–tibia feedback system

Habituation and sensitization are commonly observed during repeated stimulation in sensory and sensorimotor systems and are well described from the behavioural to the neuronal level (for reviews, see Thompson and Spencer, 1966; Peeke and Herz, 1973a,b; Kandel and Schwartz, 1982; Johnen and Schnitzler, 1989). They are central neural processes. According to Groves and Rebec (1988): "Habituation is defined as a decrease in response to a repetitive stimulus and may therefore be regarded as a means by which organisms 'learn to ignore' repetitive but inconsequential events in the environment. Sensitization can be defined as an increase in response to a repetitive stimulus, as when an organism encounters a recurring noxious or intense stimulus".

The stimulation of the FT feedback system differs in some aspects from that used in classic habituation studies. A continuous sine-wave stimulation replaces

the repetition of separate 'habituating' stimuli; the sine-wave frequency appears to be the equivalent of the repetition frequency. Nevertheless, the basic features included in the definition of habituation (Thompson and Spencer, 1966; Thompson et al. 1973) are found in the FT feedback system (an exponential time course of response decrement and its relationship to stimulus frequency, stimulus generalization and spontaneous recovery).

The increase in gain in response to tactile stimuli represents sensitization in the FT feedback system. The response increment associated with the behavioural state of the animal, defined by this term, is well known in neural reflex pathways (Kandel and Schwartz, 1982) and at the behavioural level (Peeke and Herz, 1973a,b), but has not previously been described for proprioceptive feedback systems.

The design of stimulus programmes

Although many quantitative studies have been carried out on the FT feedback system, the distinct habituation characteristics have not been observed or recognized. Similar properties may also have been overlooked in other reflexes and feedback systems. The reason may be that, under repetitive stimulation, the response of the system does not decrease to zero but to a low, constant value. Especially during a linear system analysis, when series of sine-wave cycles with different frequencies have to be tested, the system becomes habituated at the beginning of the experiment and remains so until the end. What are measured, therefore, are features of the fully habituated system. Dishabituating stimuli are absent or occur randomly and increase the variability of the response amplitude.

The results presented here show that it is possible to decrease this variability by the design of the stimulus programme. They also demonstrate that quantitative data depend strongly upon stimulus programme parameters. The number of stimulus cycles applied and evaluated, the duration of pauses and the occurrence of dishabituating stimuli not only influence the value of gain but also the shape of the frequency–amplitude curve (Fig. 5). If the pauses between stimuli are too short (<200 s), the system does not have enough time to recover and habituation takes place very quickly. This effect will be especially strong for high stimulus frequencies, when many stimulus cycles occur within a short period. In such cases, the upper corner frequency of the amplitude–frequency curve will decrease.

Dishabituating stimuli are difficult to avoid during an experiment, especially as the threshold for these stimuli decreases with the length of time that the animal is left undisturbed. Therefore, to measure responses in a defined state of behaviour, it is easier to use the dishabituated than the habituated state. The animal must be dishabituated before each stimulus and the response amplitudes of the cycles at the beginning of the stimulation must be evaluated. To measure the habituated condition of the system requires repetitive stimulation (with an equal number of stimulus cycles) for each tested frequency and the evaluation of defined cycles at the end of the stimulation. To choose the right parameters (length of pauses,

number of cycles applied and evaluated, etc.) for such a stimulus programme, the habituation characteristics of the system under investigation must be known.

The femur–tibia feedback system under natural conditions and the possible function of habituation

It is difficult to prove in which state, dishabituated or habituated, a proprioceptive feedback system operates under natural conditions. Nevertheless, the fully habituated condition – often measured during long-lasting experiments with repetitive stimuli and short pauses – does not seem to be the normal condition of the FT feedback system of a stick insect. On the contrary, many situations in which the stick insect must resist passive movements will be caused by, or be combined with, a disturbance (e.g. by a predator or another animal). These stimuli will lead to a sudden increase in gain, resulting in strong resistance reflexes and a strong depression of active movements (*flexibilitas cerea*, Bässler, 1972*b*), an appropriate reaction under these conditions.

In nature, repetitive stimuli to the fCO will normally arise from two sources. They can arise from outside, when an animal is hanging on leaves or branches swinging in the wind. Habituation causes the animal to decrease the gain rather than to resist these stimuli fully. This is relevant, as the stick insect should not spend its energy resisting these movements, but must either move with them or change its resting place. The frequency of such oscillations can cover a wide range, depending on the resonance frequency of the external system (leaves, branches). The broad frequency range of habituation indicates its significance for this purpose. Repetitive stimuli to the fCO can also arise from oscillations caused by instability of the system itself (see below).

Gain control between inefficiency and instability

The parameter gain must be adjusted very carefully in a feedback system to provide an effective but stable reaction. Bässler *et al.* (1974) showed by means of a linear system analysis that the FT feedback system might be close to instability for high values of gain, but instability of the closed-loop system as demonstrated in Fig. 7 has not been observed up to now. Two experiments confirmed their hypothesis. A linear (Bässler *et al.* 1974) or rotatory pendulum (Pfeiffer, 1989) was coupled to the tibia to increase its moment of inertia. The feedback system became unstable and produced regular oscillations of the tibia at high amplitudes and with a frequency of 2–4 Hz, the resonance frequency of the system. After a period of constant oscillations, the amplitude decreased to zero. In the artificially closed feedback system, with an electronic delay of 50–200 ms in the feedback signal path, similar reactions were observed (Weiland *et al.* 1986).

The occurrence of stable oscillations due to an increase in the gain is not possible in a linear system. Above a critical value of the gain, the amplitude of oscillation increases from cycle to cycle and quickly reaches its physical limit. Bässler *et al.* (1974) explained the stable oscillations by proposing unknown nonlinearities. They found an almost constant value of the gain for stimulus amplitudes of 15, 55

and $100\,\mu m$. This is in contradiction to the results presented here, and might be explained by the large variations in their original data (compare Figs 1, 2 and 3; Bässler *et al.* 1974) and by differences in the stimulus programme. The relatively high values of gain in their study result from the different ratio between fCO movement and FT angle amplitude ($100\,\mu m = 13°$, Bässler *et al.* 1974; $100\,\mu m = 20°$, middle leg, this study) used for their calculation of gain. They investigated the response amplitudes of 7 middle and 11 hindlegs, and it is unclear from which legs (possibly hindlegs) this ratio was measured.

The nonlinearity of the FT feedback system described here – the decrease in gain with increasing stimulus amplitude – explains the occurrence of stable oscillations in the experiments mentioned above. The same principle is often used in technical systems to build stable oscillators. This nonlinear function of gain *versus* stimulus amplitude is the first mechanism stabilizing the feedback system. It prevents the system from going to the limit for higher values of gain, but leads to stable oscillations. The second mechanism, habituation, brings the system back to stability. This feature of the system decreases the gain when repetitive stimuli or oscillations of the system occur. After an increase in gain above a critical value, caused, for example, by a disturbance, the stable oscillations produced by the first mechanism in combination with the decrease in gain caused by the second mechanism adjust the gain to a value at which stability is re-established. The resulting gain is the highest and most effective value that the feedback system can show. No further reduction in gain occurs when the oscillations cease. The system remains in this optimally adjusted state and the gain decreases only slightly with time.

The results from the closed-loop system show exactly the predicted behaviour: the increase in gain caused by a tactile stimulus is followed by damped oscillations at the resonance frequency, which lead to a return to stability. The data show that the gain in a feedback system is itself under a feedback-like control.

The gain control system

The dual-process theory of habituation (Groves and Thompson, 1970, 1973; Thompson *et al.* 1973) states that the strength of the behavioural response elicited by repeated stimulation is assumed to be the net outcome of the two independent processes of habituation and sensitization. While habituation takes place in the stimulus–response pathway, sensitization acts on the 'state system(s)'. The theory is based on the results of behavioural and neurophysiological studies and neuronal models.

The results presented in this paper are in accordance with the assumptions of the dual-process theory of habituation. Adaptation of that theory to the FT feedback system leads to the following conclusions. The gain of the FT feedback system is the net outcome of two processes: habituation and sensitization. Habituation can only appear in the pathway between the fCO and the extensor and flexor tibiae muscles of the leg under repetitive fCO stimulation. This means that the gain of the FT feedback systems for the six legs can exhibit different, independent values,

and that habituation of the FT feedback system in one leg should not influence the gain in the feedback systems in other legs – a statement to be tested in further investigations. Nevertheless, the gain of all proprioceptive feedback systems in all legs will be increased by sensitization, a property of a 'state system' that determines the behavioural state of the animal. Stimuli from all different sources can act on this system. It increases the gain of the stimulus–response pathway of all the legs at the same time and counteracts habituation.

Stimulus generalization of habituation (see Groves and Thompson, 1973), present in the FT feedback system in the stick insect, shows that, for the different stimulus frequencies, common habituation elements are used. Therefore, these elements, probably interneurones or motoneurones and their connections, that change their transduction properties during habituation, should have a broad bandwidth for sinusoidal fCO stimuli, an assumption yet to be tested with intracellular recordings from the central nervous system of the stick insect. These predictions could be tested by further studies on the neuronal basis of gain control.

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