

CENTRAL CONTROL OF AN INSECT SENSORY INTERNEURONE

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INTRODUCTION

A previous paper (McKay, 1969) described the response characteristics of the large auditory T fibre of the tettigoniid *Homorocoryphus*. This neurone is both frequency-sensitive and amplitude-sensitive, and does not respond to the species song. It responds preferentially to short, high-frequency sounds, which, together with the large size of the axon, suggests that it may function as a warning neurone. Recent work by Rowell & McKay (1969) indicates that the responsiveness of insect sensory interneurons can be controlled by auxiliary inputs, often inhibitory, which are central in origin. Rowell (1964) found that lesions in the central nervous system of *Schistocerca* increased the frequency of a behavioural reflex controlled by the prothoracic ganglion. Increased responsiveness was correlated with a decrease in the input to the ganglion, and increased output from it (Rowell, 1969), indicating that inhibitory influences had been interrupted by the lesions. Rowell & McKay (1969) found that disconnecting the head ganglia of *Gastrimargus* from the rest of the central nervous system markedly increased the responsiveness of the alpha auditory interneurone, while subsequent lesions had little or no effect.

Habituation is also controlled by central factors. Habituation is the waning of a response to a repeated stimulus, and is seen in many vertebrate sensory interneurons (Horn, in press) and in a growing number of invertebrate sensory interneurons (Horridge, Scholes, Shaw & Tunstall 1965; Horn & Rowell, 1968; Rowell & McKay, 1969). Habituation is characterized by stimulus specificity, recovery after a time lapse, and sometimes by dishabituation by another modality of stimulus (Horn, 1967). It is an important property of interneurons in that it enables repetitive, irrelevant stimuli to be ignored, and novel stimuli to be recognized easily. Visual units in the tritocerebrum and ventral nerve of *Schistocerca* (Horn & Rowell, 1968; Rowell & Horn, 1968; Palka, 1967) show complex habituation phenomena which may last for several hours. Similar characteristics have been observed in the alpha auditory interneurone of *Gastrimargus* (Rowell & McKay, 1969). The habituation rate of this interneurone is decreased by the removal of the head ganglia, but other lesions to the central nervous system have very little effect.

This paper describes the effect of lesions to the central nervous system on the responsiveness and habituation rate of the large auditory T fibre of the tettigoniid, *Homorocoryphus*. The results suggest that the response level of the interneurone is controlled by an inhibitory input or inputs, which originate within the posterior

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thoracic ganglia and act synaptically within the prothoracic ganglion. Habituation of the T fibre is found to be less complex than habituation of other insect interneurons described to date. The rate of habituation is also under the control of central factors, which may be different from those which affect the response level of the interneurone.

MATERIALS AND METHODS

The response of the T fibre to repetitive acoustic stimuli was investigated using methods described in a previous paper (McKay, 1969). The stimulus train consisted of 20 pulses of 10 kc/s, each of 40 msec duration. The amplitude of each pulse was +94 dB (0 dB = 0.0002 dyn/cm²). Response curves of the interneurone were recorded in the neck connectives by means of silver wire electrodes. Recordings were made

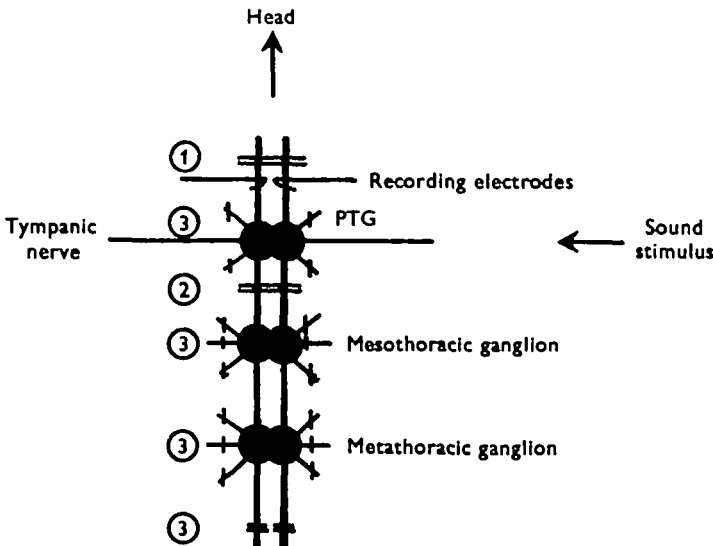


Fig. 1. Diagram of the central nervous system of *Homorocoryphus*, showing the lesions made in the experiments described. (1) Lesion of the neck connectives, (2) Lesion of the pro-mesothoracic connectives. Lesions 1 and 2 isolate the prothoracic ganglion from the rest of the central nervous system. (3) Lesions made to de-afferent the thoracic ganglia. In some experiments, recordings were made from the intact animal, and then after the neck connectives had been cut. A final recording was then made after the prothoracic ganglion had been disconnected from the rest of the central nervous system by cutting the pro-mesothoracic connectives. In other experiments, the order of the lesions was different: the pro-mesothoracic connectives were cut after a recording had been obtained from the intact animal, and a final recording was obtained after the prothoracic ganglion had been isolated by cutting the neck connectives. In experiments in which the thoracic ganglia were de-afferented, all inputs to the thoracic ganglia except the tympanic nerves, were cut.

from the intact animal, and then after the head ganglia had been disconnected by cutting the neck connectives. A final response curve was recorded from each animal after the prothoracic ganglion had been completely isolated from the rest of the central nervous system by cutting the pro-mesothoracic connectives. In some animals, the order of lesions was as follows: the pro-mesothoracic connectives were cut after recording from the intact animal, and the prothoracic ganglion was subsequently isolated from the rest of the central nervous system by cutting the neck connectives (Fig. 1).

RESULTS

The results presented below were obtained from a total of 20 adult insects. No differences were observed between the responses of male and female insects.

When a train of pulses is played to the intact animal, the response to each pulse is phasic and shows little habituation. The response to the first pulse of the train is largest, and subsequent responses decline to a plateau but never reach zero, even when the initial response level is low (Fig. 2). The T fibre response recovers completely

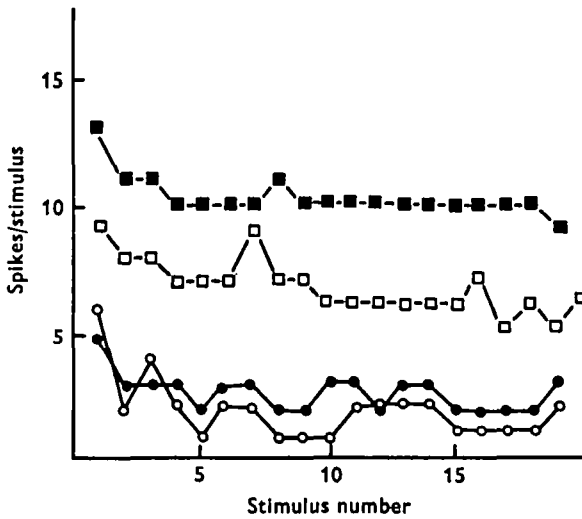


Fig. 2. Response level and habituation curves for different lesion conditions. ○—○, intact; ●—●, neck connectives cut; □—□, pro-mesothoracic connectives cut; ■—■, prothoracic ganglion isolated.

within 10 min. Response curves were obtained for the lesion conditions described above (see also Fig. 1). The response decrement (Fig. 2) was seen to be approximately linear, unlike the exponential decay seen in the alpha neurone of *Gastrimargus* (Rowell & McKay, 1969). Accordingly, the data for each trial was fitted to regression equations of the type $y = a + bx$, where y = number of spikes/response, and x = stimulus presentation number. The correlation coefficient, r , was also obtained for each of these fits, and gives a measure of how closely the assumption that the decrement is linear is justified. The results (Table 1) suggest that this assumption is a reasonable one. The parameters a and b are measures of the response level and habituation rate respectively (see Rowell & McKay 1969, for further discussion). The values of a , b , and r were calculated for the response curves obtained from the intact animal, and for all lesion conditions, i.e. with the neck connectives cut, the pro-mesothoracic connectives cut, and the prothoracic ganglion isolated from the rest of the central nervous system. These values are set out in Table 1. The significance of difference between the values of both a and b for different lesion conditions was tested by the Walsh test for matched pairs, the results from individual animals supplying the pairs.

Table 1. *The values of a, b and r as calculated from the regression equation $y = a + bx$, where $y = \text{response}$ and $x = \text{stimulus number}$ (a and b are measures of response level (intercept on y axis) and habituation rate (slope of the line) respectively. r is the correlation coefficient.)*

Animal	Intact nervous system			Neck connectives cut			Pro-mesothoracic connectives cut			Prothoracic ganglion isolated		
	a	b	r	a	b	r	a	b	r	a	b	r
D2	4.2	-0.20	-0.46	—	—	—	2.3	-0.13	-0.38	12.8	-0.41	-0.83
D6	—	—	—	—	—	—	—	—	—	12.0	-0.18	-0.61
D7	—	—	—	5.6	-0.41	-0.56	—	—	—	6.8	-0.10	-0.36
D8	5.3	-0.03	-0.08	3.8	-0.03	-0.11	—	—	—	8.8	-0.29	-0.39
D10	2.2	-0.06	-0.50	—	—	—	7.9	-0.24	-0.51	7.1	-0.23	-0.68
D11	2.5	-0.15	-0.55	3.1	-0.04	-0.40	—	—	—	6.3	-0.15	-0.87
D12	2.2	-0.05	-0.17	—	—	—	8.9	-0.33	-0.85	11.6	-0.18	-0.78
D14	2.7	-0.05	-0.11	—	—	—	6.6	-0.14	-0.42	10.7	-0.33	-0.48
D15	1.9	-0.17	-0.44	—	—	—	4.7	-0.13	-0.25	3.1	-0.80	-0.37
D16	3.8	-0.14	-0.41	—	—	—	5.2	-0.72	-0.19	6.5	-0.12	-0.58
D17	3.6	-0.23	-0.59	1.8	-0.06	-0.38	—	—	—	2.6	-0.10	-0.70
D18	3.1	-0.10	-0.44	3.2	-0.16	-0.60	—	—	—	6.3	-0.35	-0.72
D19	4.0	-0.05	-0.18	5.6	-0.22	-0.61	—	—	—	7.2	-0.05	-0.51
D25	—	—	—	5.0	-0.36	-0.60	—	—	—	9.7	-0.26	-0.59
D26	—	—	—	3.3	-0.18	-0.49	—	—	—	14.5	-0.43	-0.72
D27	—	—	—	—	—	—	—	—	—	14.5	-0.06	-0.27
D28	—	—	—	2.2	-0.01	-0.50	—	—	—	7.6	-0.19	-0.54
D29	—	—	—	3.8	-0.06	-0.28	—	—	—	13.1	-0.16	-0.61
D48	—	—	—	2.8	-0.09	-0.41	—	—	—	5.9	-0.26	-0.52
D54	—	—	—	4.8	-0.28	-0.61	—	—	—	3.1	-0.40	-0.18
Mean	3.3	-0.12	-0.40	3.8	-0.16	-0.50	5.9	-0.29	-0.50	8.5	-0.25	-0.60

(1) Change in habituation rate, *b*

There is no significant difference in the rate of habituation between the intact animal and the animal with neck connectives cut. There is, however, a significant increase ($P \leq 0.05$) in the rate of habituation in the animal in which the pro-mesothoracic connectives have been cut. When the prothoracic ganglion is isolated by cutting the

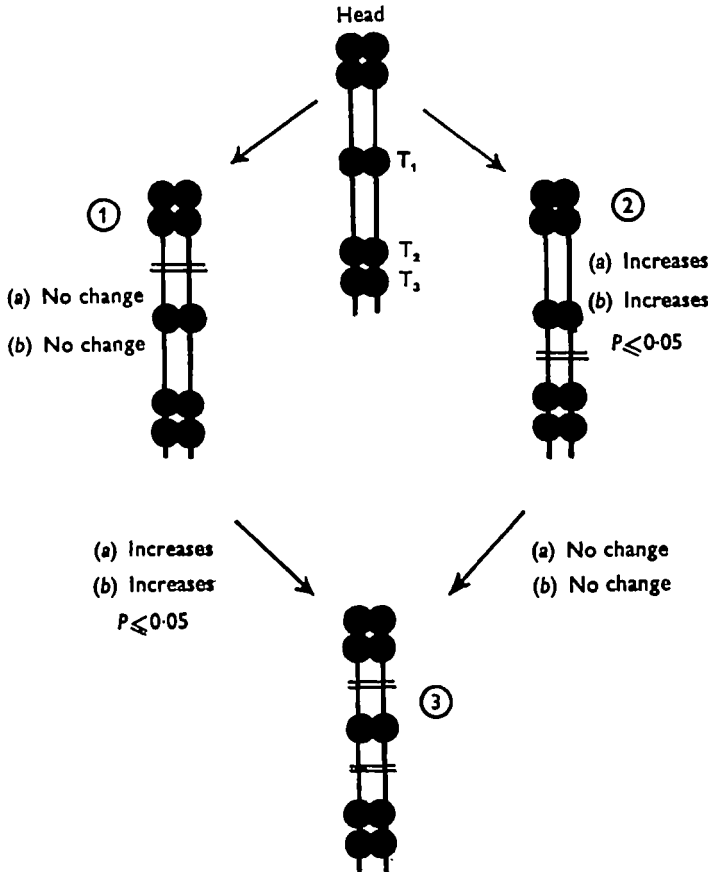


Fig. 3. The effects of lesions to the central nervous system on the response level, *a*, and the rate of habituation, *b*, of the T fibre. When the neck connectives are cut (1), there is no change in the response level and the rate of habituation. When the pro-mesothoracic connectives are cut (2), there is an increase in the response level and in the rate of habituation. When the prothoracic ganglion is isolated by cutting the pro-mesothoracic connectives in a preparation in which the neck connectives have already been cut (1 → 3), there is an increase in the rate of habituation and response level. When the prothoracic ganglion is isolated by cutting the neck connectives in a preparation in which the pro-mesothoracic connectives have already been cut (2 → 3), there is no change in the response level or rate of habituation. This suggests that the response level and rate of habituation of the T fibre are kept low in the intact animal by an inhibitory input or inputs from the posterior thoracic ganglia to the prothoracic ganglion. T₁ = Prothoracic ganglion; T₂ = mesothoracic ganglion; T₃ = Metathoracic ganglion.

pro-mesothoracic connectives in an animal in which the neck connectives had already been cut, there is a significant increase in the rate of habituation ($P \leq 0.05$) over the condition in which the neck connectives only have been cut. When the prothoracic

ganglion is isolated by cutting the neck connectives in an animal in which the pro-mesothoracic connectives have previously been cut, there is no significant change in the rate of habituation. These results are shown diagrammatically in Fig. 3. The intrinsic rate of habituation of the T fibre, indicated by the values of b in the isolated ganglion, is seen to be low (Table 1). The lesion experiments indicate that the rate of habituation is reduced still further in the intact animal by inputs to the prothoracic ganglion which arise within the posterior thoracic ganglia. The head ganglia appear to exert no effect on the rate of habituation.

(2). *Changes in response level, a*

The response level of the T fibre depends on the frequency and amplitude of the stimulus (McKay, 1969). The present results show that, when these parameters of the stimulus are held constant, the response level is affected by inhibitory inputs from within the central nervous system. There is no significant change in the response level (parameter a) over that in the intact animal when the neck connectives are cut. There is a significant increase ($P \leq 0.05$) from the intact response level when the pro-mesothoracic connectives are cut. When the neck connectives are cut in a preparation in which the pro-mesothoracic connectives have already been cut, there is no further increase in the response level. When the prothoracic ganglion is isolated in an animal in which the neck connectives have been cut, by cutting the pro-mesothoracic connectives, there is a significant increase in the response level ($P \leq 0.05$, see Fig. 3).

These results indicate that, in the intact animal, the response level is maintained at a low level by an inhibitory input or inputs to the T fibre in the prothoracic ganglion. These inputs arise within the posterior thoracic ganglia. This data shows that the head ganglia do not affect the response level of the T fibre. These inhibitory influences are not mediated by any sensory input, as in *Locusta* (Yanagisawa, Hashimoto & Katsuki, 1967). When the thoracic ganglia are de-afferented (Fig. 1) there is no change in the response level with reference to that in the intact animal, and an increase in level does not occur until the pro-mesothoracic connectives are cut. This indicates that the inhibitory influences arise within the central nervous system, within the posterior thoracic ganglia, and that they are not a consequence of extratympanal sensory input.

(3). *Habituation and response level*

The rate of habituation is loosely correlated with the response level. There is a small increase in the rate of habituation with the increase in response level, but the calculated regression ($r = -0.56$) indicates that the correlation of the two characters is not a close one.

DISCUSSION

Habituation of insect sensory interneurons to repetitive stimuli has been observed by several workers (Horridge, *et al.* 1965; Palka, 1967; Horn & Rowell, 1968; Rowell & McKay, 1969), and is important in that it provides a mechanism whereby an animal can ignore irrelevant stimuli, and respond more effectively to novel occurrences in the environment. It differs in this respect from the visual units of the tritocerebrum of *Schistocerca* (Horn & Rowell, 1968) and the alpha auditory interneurone of *Gastrimargus* which habituate rapidly. The slow habituation rate of the T fibre may be correlated

with its probable function as a warning neurone (McKay, 1969). The rate of habituation of the T fibre is increased by lesion of the pro-mesothoracic connectives, suggesting that, in the intact animal, the rate of habituation is maintained at a low level by accessory inputs from the posterior thoracic ganglia to the prothoracic ganglion. This may be contrasted with the situation which is seen in *Gastrimargus* (Rowell & McKay, 1969), where the habituation rate is high in the intact animal and is decreased by disconnecting the head ganglia from the rest of the CNS by lesion of the neck connectives. The rate of habituation of the T fibre and the alpha neurone are, therefore, controlled

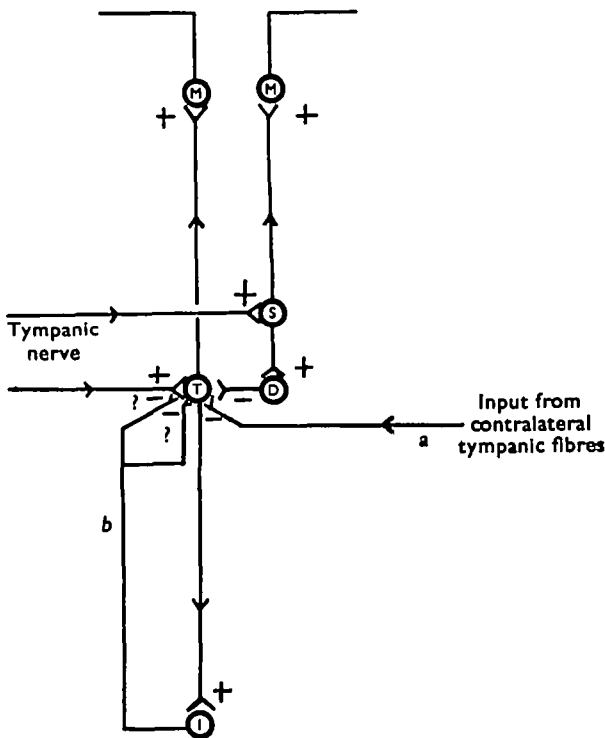


Fig. 4. Neuronal connexions of the T fibre. The T fibre (shown here diagrammatically with the cell body as the point of impulse initiation) is driven by the input from the ipsilateral tympanic nerve. The response of the T fibre to any given auditory stimulus is modified by (a) an inhibitory input from the contralateral tympanic nerve fibres, and (b) an inhibitory input from the posterior thoracic ganglia. The magnitude of the latter input may be controlled by the activity in the posterior branch of the T fibre. The inhibitory input from the interneurone, I, to the T fibre may act presynaptically or postsynaptically. The T fibre does not respond to the species song and is inhibited by a smaller fibre, S, which does respond to the species song (J. M. McKay, 1969). Inhibition may occur via an interneurone, D. Both the T and S fibres are thought to drive behavioural responses via the motoneurons, M.

by accessory inputs to the ganglion in which impulse initiation occurs. The origin and effect of these accessory inputs is different for the two neurones. It is suggested that the rate of habituation of any interneurone is a result of selection, and that the habituation rate reflects the role of the neurone in controlling the behaviour of the animal. Neurones with a rapid rate of habituation will be concerned with the recognition of

novel stimuli, e.g. the alpha neurone of *Gastrimargus* and visual units of *Schistocerca*, while those with a low rate of habituation, e.g. the T fibre of *Homorocoryphus*, will be concerned with the recognition of repetitive stimuli which cannot be ignored. The T fibre of *Homorocoryphus* seems to serve as a warning neurone. The modification of the rate of habituation of a neurone by accessory inputs suggests that individual neurones do not have sufficient plasticity to maintain extreme rates of habituation.

The response level of the T fibre is also reduced in the intact animal by an inhibitory input or inputs to the prothoracic ganglia from the posterior thorax. The head ganglia are seen to have no effect on the response level of the T fibre. The T fibre sends a descending branch from the prothoracic ganglion to the posterior thorax, and discharge by this fibre may initiate a feedback loop which controls subsequent inhibitory output from the pterothorax (Fig. 4) maintaining the response level of the T fibre at a low level. Stabilization of the T fibre response at a low level would prevent overloading of the system at high stimulus amplitude, which could lead to the confusion of pulses and loss of directional information. The inhibitory input maintains a phasic response, thus rapid pulses are not confused.

The observed correlation between response level and habituation rate suggests that a further function of feedback stabilization of the response at a low level may be to minimize habituation. This is in keeping with the hypothesis that habituation reflects a response decrement brought about by the depletion of transmitter substances at the active synapses (Bruner & Tauc, 1966; Horn, 1967). The correlation between the response level and rate of habituation, although quantitatively valid, is not particularly close ($r = 0.56$). This suggests that these parameters of the response are either non-linearly related by common synaptic events, or, alternatively, they are controlled by different accessory inputs from the posterior thorax. The synaptic system affecting the T fibre is complex (McKay, 1969), and while changes in response level and habituation are a result of changes at one synapse only, it is more likely that changes in sensitivity at several synapses are involved. The inhibitory input from the posterior thorax to the prothoracic ganglion is not influenced by any peripheral input to the central nervous system, as demonstrated by experiments in which the thoracic ganglia were de-afferented. The inhibitory input to the prothoracic ganglion may act pre-synaptically or postsynaptically, but presynaptic inhibition would give the fine control required of a negative feedback system.

The auditory systems of the Orthoptera are seen to be far from simple, as was once thought. The response properties of each interneurone may be correlated with the role, or probable role, it plays in controlling the behaviour of the animal, and in the case of the T fibre the properties are in part maintained by synaptic systems within the central nervous system, which are independent of peripheral inputs.

SUMMARY

1. The T fibre habituates little to a series of sine-wave pulses, and recovery is complete within 10 min.
2. Both response level and rate of habituation are increased when the posterior thoracic ganglia are disconnected from the prothoracic ganglion. This indicates that response level and rate of habituation are maintained at a low level in the intact

animal by an inhibitory input or inputs to the prothoracic ganglion, which arise within the pterothorax. These inputs are entirely central in origin, and are independent of extratympanal sensory input.

3. The function of the posterior branch of the T fibre to the posterior thorax may be to initiate a feedback loop which is completed by the input from the posterior thorax. It is suggested that such feedback stabilization of the response level would reduce loss of directional information at high stimulus amplitude, preserve discrimination at high stimulus repetition rates and reduce habituation. The implications of these findings for a warning neurone system are discussed.

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