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STUDIES ON THE NEURAL PROCESSING OF CONSPECIFIC SONGS IN THE CRICKET TELEOGRYLLUS OCEANICUS (LE GUILLOU).

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January 1987.

I hereby declare that this thesis is my own work, except where the contrary is specifically indicated. No other registration for an award of either the CNAA or any University occurred during the period of this research programme.

January 1987.

L.A. Harrison.

CONTENTS.

Advanced studies undertaken in connection with this programme of research included attending the M.Sc. course; Neurophysiological Basis of Behaviour at the City of London Polytechnic, and attendance of seminars and conferences.

Section 1.1 forms the basis of a paper which has been submitted for publication.

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ABSTRACT.

STUDIES ON THE NEURAL PROCESSING OF CONSPECIFIC SONGS IN THE CRICKET TELEOGRYLLUS OCEANICUS (LE GUILLOU).

Lesley Anne Harrison.

This study involved investigations into the neural processing of auditory information by the Australian field cricket Teleogryllus oceanicus in relation to the identification of its conspecific songs. T. oceanicus males produce three song types related to three different behavioural strategies. Although each of the three songs have very similar frequency spectra their temporal patterns are very different and highly complex, particularly the courtship song. Song type recognition therefore is likely to be based on neurones capable of producing an accurately coded response to the song patterns. Using extracellular and intracellular recording techniques, neurophysiological and neuroanatomical investigations were carried out in order to identify auditory interneurones in the pro-thoracic ganglion capable of coding for the temporal patterns of the songs. Two examples of the ascending class of neurones were identified and shown to respond to the conspecific song patterns: ANC, which coded the temporal pattern of the calling song and ANA, which produced a correlated response to the temporal pattern of the courtship song. Further investigations showed that as a result of the frequency content, syllable rate and intensity of the song, it was the integration of excitatory and particularly inhibitory inputs that allowed ANA to code for the courtship song.

To identify other neurones involved in this pathway the origin of the inhibitory input was investigated. The local bilateral omega neurone, ON1, was thought to mediate the inhibition. Current manipulation experiments which involved simultaneous extracellular and intracellular recordings from ANA and ON1 respectively were carried out. However, these experiments showed no evidence for the existence of effective synapses between these two cells.

A second type of omega neurone was identified, ON2. Although this neurone was shown to be non-spiking its response was correlated with the temporal patterns of the songs. Preliminary investigations were carried out on examples of descending and through neurones.

FIGURE PAGE NUMBERS.

CHAPTER 1.

INTRODUCTION.

1.1 The Songs of Insects.

Sound is the major means of intra specific communication for many insect species. While visual cues require the exposure of the emitter to predator attention, sound can be broadcast from deep undergrowth or at night. Sound can be patterened far more effectivley (and therefore carry more information about the identity and state of the emitter over greater distances) than chemical signals which are disrupted by air currents. Each modality is constrained by its own particular set of physical requirements and sound is no exception: there are constraints on the frequency content and temporal characteristics that can be generated by the emitter; complex environments can impose limitations on the integrity of the signal by disrupting time cues and absorbing frequencies preferentially; and the receiver faces major problems in the analysis of the signal structure and in the localisations of the sound source. However, despite such physical and environmental limitations, sound communication in insects has evolved to a high degree of sophistication with many species overcoming the demands of their particular niches.

The sounds produced range from the low frequency, substrate-borne vibrations of small insects such as the landbugs, to the complex, more widely known air-borne songs of the Orthoptera. With very few exceptions (e.g. Amblycorypha uhleri; Walker and Dew 1972) insects do not

show the frequency sweeps so common in bird songs; the frequency spectrum in crickets is usually that of a fundamental and its harmonics (Nocke 1972), whereas grasshoppers and most bush crickets have a broad band, almost "white noise" song (Sales and Pye 1974). In most Orthopteran species the temporal patterns of the songs appears to be of major importance both in terms of species recognition and song type recognition.

Species recognition relies initially on the production and reception of a calling (or proclamation) song. The main function of this song is to attract the adult females prior to courtship. However behavioural experiments have shown that male crickets are also attracted by their conspecific calling song (Ulagaraj and Walker 1973, Pollack 1982). In crickets and bushcrickets this behaviour allows the males to maintain regular dispersion within a group and so avoid unecessary conflicts (Campbell and Shipp 1979; Latimer 1980; Schatral, Latimer and Kalmring 1985). This diphasic role of a calling song is well known in Anurans (Littlejohn and Harrison 1985) particularly in the tree frog Eleutherodactylus coqui where the song has two components of which the "co" part is involved in male-male interactions and the "qui" component is used to attract females (Nairns and Capranica 1976) During Orthopteran and Anuran communication these songs are repeated for long periods but the temporal pattern remains constant. Repeated production of phrases of sound is, of

course, information transfer with a great deal of redundancy, but it improves the likelihood of sucessful communication. In other words the fixed action pattern of the song is more likely to release the appropriate behaviour.

An intrusion into the territory of one cricket by another male will usually result in the singer switching from its calling song to its aggressive song. Bushcrickets will defend their singing positions on shrubs by aggressive interactions with other males (Morris 1971; Schatral, Latimer and Broughton 1984). Crickets set up dominance hierarchies based on escalating male-male interactions and often the production of the aggression song is sufficient to determine the outcome of the conflict (Alexander 1961, Loher and Rence 1978). Approach by a female on the other hand, results in a switch from calling to courtship song which produce quite different behavioural characteristics in the female. Release of appropriate behaviour in the receiver therefore requires recognition of the species song and the song type. Many investigations are being carried out at present to try and determine how insects recognise and distinguish between inter- and intra-specific sounds.

The aim of this introduction is to bring together aspects of these areas of research and to try and provide an overall understanding of the roles of sound in the lives of insects.

1.1.1. Sound Production.

The production of sound which allows effective communication requires insects to overcome a number of basic physical problems.

(1) The generated sound must be of a sufficiently high intensity to propagate over a reasonable distance from the source . In general, the greater the intensity, the greater the catchment area for that singer. It may be that there is an evolutionary advantage in choosing a male with a more intense song since this may be correlated with a larger body size. This is the situation found in the North American toad Bufo americanus. In this species the intensity of the mating calls are positively correlated with the body size of the toad (Gerhardt 1975).

(2) The frequencies produced must be consistant with the size of the radiating structure: efficient sound radiation occurs when the diameter of the source is equal to, or greater than, the wavelength of the sound produced. In most insects, sound is radiated from both surfaces of the vibrating structure. However, these two sound waves are 180° out of phase with each other; compression on one side is accompanied by rarefaction on the other. If the diameter of the source is smaller than the wavelength, the opposite sound waves will pass around the radiator and cancel each other on the plane perpendicular to the direction of the sound. This phenomenon is known as acoustic short-circuting

(Michelsen 1983), and it places a lower limit on the frequencies which can be generated. The problem is compounded by the fact that the basis of sound production in all insects is, ultimately, muscle contraction. The maximum known rate of muscle contraction, found in the bushcricket species Neoconocephalus, is about 200Hz (Josephson and Elder 1968). 200Hz has a wavelength of about 170cm; this compares to a wing diameter of around 2cm. Clearly a case for acoustic short-circuting and an inefficient radiation of sound. In insects where the muscle contraction rate is about 50Hz the problem is even greater.

(3) In theory therefore, insects should only be able to effectively emit short wavelength (high frequency) sounds. The physics of environmental sound propagation is highly complex and depends upon a number of factors (Michelsen 1983). As a general rule however, low frequencies (long wavelengths) are transmitted more effectively than high frequencies (short wavelengths) for the following reasons. Sound waves will spread out from the sound source in all directions, and are attennuated over distance due to geometrical spreading. All frequencies are attenuated on the basis of the inverse square law - a factor of 6dB per distance doubled. Additional attenuation occurs because of absorption by the medium and high frequencies are attenuated more rapidly than low frequencies. For airborne-sound the situation is exacerbated if the air is humid - which is probably why bats do not fly in fog. In

environments consisting of dense vegetation, high frequencies often have wavelengths consistent with the diameters of leaves and branches. Excess attenuation of high frequencies therefore occurs because of scattering or diffraction and reflection of the propagating wave. Still more high frequency energy is lost as absorption by the leaves when they are set into vibration by the passage of sound (Martens and Michelsen 1981).

In order to overcome these constraints (to a greater or lesser degree) insects have evolved some common strategies to increase both the range and the intensity of their communication sounds. Others have evolved far more specialised aids to communication.

(i) Common strategies.

(a) Stridulation

Within the Orthoptera the structures which generate and radiate the sound are arranged in two ways. In the field crickets (Grylloidea) and bush crickets (Tettigonioidea) they occur on the wings (an elytro-elytral system); in the grasshoppers (Acridoidea) the wings and the hindlegs are involved (a femero-elytral system). Basically however the mechanism is the same in both cases and is a means for frequency multiplication: the increasing of the rate of muscle contraction to a frequency consistent with effective radiation, by means of a file and plectrum system (Sales and Pye 1974, Elsner 1985).

In bushcrickets (Fig. 1.1(i)) and crickets (Fig. 1.1(ii)) the file (a row of sclerotized teeth developed from a series of folds around a vein) is situated on the under surface of the forewing or tegmen. The edge of the other tegmen has an area of thickened cuticle which forms the plectrum. During wing closure (one contraction of segmental muscles) the plectrum strikes a series of teeth on the file, each impact causing a vibration containing a range of frequencies. The whole movement sets up a train of vibrations with a fundamental frequency determined by the tooth impact rate (Koch 1980). However the sound produced is determined not only by the tooth impact rate but also by the properties of a sound radiator which is associated with the stridulatory mechanism. In bushcrickets the sound radiator is the mirror frame situated on the right tegmen (Fig. 1.1(i)). It consists of rigid, sclerotized wing veins which more or less surround the mirror membrane (Latimer 1980). The membrane itself is not involved in sound radiation (Bailey 1970). In crickets the sound radiator is a triangular area of the cuticle traversed by four veins and called the harp (Fig 1.1.(ii): Nocke 1971). There is a harp on each tegmen but the main radiator is that found on the left.

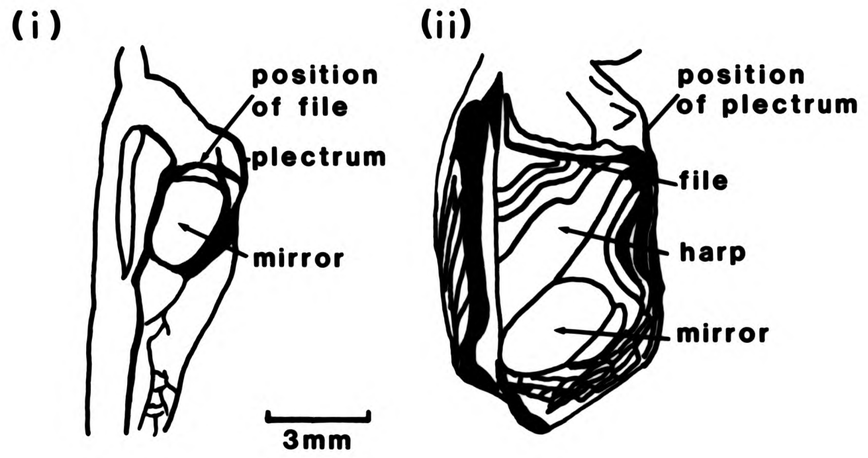
The frequency content of the sound emitted depends on the relationship between the tooth impact rate and the frequency of natural vibration of the radiator. If the tooth impact rate is in phase with the oscillations of the

Fig. 1.1.

The stridulatory apparatus on the right tegmen of,

(i) the bushcricket Platycleis intermedia and

(ii) the cricket Gryllus bimaculatus



radiator, the whole movement of closure sets up a train of continuous waves with very little decay between each tooth strike. This is a resonating system and results in the production of an almost pure tone. Such resonating systems occur in crickets and in some bushcrickets (e.g. Ruspolia species; Bailey and Broughton 1970). Whereas Ruspolia emit sounds in the 12-15kHz range the songs of most cricket species are centred on 4-5kHz. This is quite a large wavelength to be produced by such a small insect and some degree of acoustic short-circuting would be expected. The enhanced intensity produced by a resonating system compensates for this problem and allows efficient emission of sound.

Most bushcrickets however are non-resonant, in that the tooth impact rate is well below the frequency of natural vibration of the mirror frame. Thus, the pulse produced by each tooth impact decays before the onset of the next. This rapid damping of each vibration results in a sound with a broad frequency band the dominant frequency of which is that of the frequency of mirror frame vibration, (Broughton, Samways and Lewis 1975; Sales and Pye 1974).

The stridulatory mechanisms of acridid grasshoppers appear to have evolved twice. In the sub-family Gomphocerinae, the stridulatory file is on the femur and the plectrum is formed from one of the wing veins. The situation is reversed in the sub-family Oedipodinae where

the stridulatory pegs are on a wing vein and the plectrum on the hind legs. Functional stridulatory systems occur on both sides so that there are two sound sources (Elsner and Popov 1978). Sound production is non-resonant. No specialised area of the wings or hind legs have been shown to be resonant.

(b) Tymbal mechanisms.

The use of tymbal mechanisms to produce sound has been highly developed by the cicadas. These insects have a pair of tymbal organs situated on each side of the first abdominal segment. Each tymbal consists of a series of ribs and a sclerotized tymbal plate. The contraction of large tymbal muscles against this plate causes the ribs to buckle and the whole tymbal to collapse with a snapping action, similar to the buckling of a metal plate. This movement produces sound (Simmons 1977). Due to the elastic properties of the cuticle the tymbal membrane rapidly regains its original shape after the contraction. However, the faint click produced by this movement is not thought to be part of the sound used for communication.

In the Australian bladder cicada, Cystosoma saundersii, the sound is made up of two distinct pulses caused by the buckling of the first rib and then the buckling of the rest (Simmons and Young 1978). The number and shape of the ribs determines the number of pulses produced by each contraction and they vary from species to

species. Abricta sp. and Magicicada sp. both have ten highly sclerotized and prominent ribs on each tymbal. During contraction, the buckling of these produces ten pulses. This is therefore another example of rate multiplication in that one muscle contraction can result in a series of pulses. The cicadas also have a resonating system. The male is able to trap air inside sacs in its abdomen which it then extends out beyond the wings. This acts as a resonator to emphasise the natural vibration frequency of the tymbal which is determined by the construction of its ribs and plate. During singing the intensity of the sound produced is further increased by the tonic contraction of a tensor muscle which stiffens the tymbal (Simmons and Young 1978; Fletcher and Hill 1978). These tymbal sounds generally have quite low carrier frequencies ranging from 800Hz to 6-10kHz and are only produced by the male. However, most species also have a higher frequency wing clicking sound produced by both the male and female which may be used in longer range communication (Popov 1981).

The extent to which an emitter performs in a resonant manner can, in all cases, be determined by calculating the Q or quality value of the sound. It is based on the determination of the relative amplitudes of the frequencies that are radiated. It is calculated by dividing the frequency (in Hz) which has the maximum amplitude, by the difference (in Hz), between the

frequencies above and below this frequency, that are either 3dB (Q3dB) or 10dB (Q10dB) down in amplitude. With a narrow bandwidth (resonance) the difference is small and therefore the Q is high: the impact energy is dissipated slowly, with long rise and fall times. In contrast non-resonant systems have low Q values: the energy is dissipated rapidly over a wide range of frequencies (Bennet-Clark 1975; Sales and Pye 1974).

(c) Vibration.

The efficiency of sound radiation in very small insects is extremely low: the coupling of the sound radiator to air is poor and only improves substantially at high ultrasonic frequencies where propagation distance is small. An alternative to increasing the energy necessary for high intensity airborne sound generation is to transmit the sound via another medium. More dense media than air allow the emitted sound to be radiated more effectively because of the increased propagation velocity of the sound wave (c) and the density of the medium (ρ). In air, c = 340m/s whereas in water c = 1500m/s. In air, ρ = 1.2kg/m^3 and in water, ρ = 1000kg/m^3. Therefore the product, c , is about 3500 times larger in water than in air.

Since I, the sound intensity of the wave is related to c by the equation

$$I = \frac{P^2}{c\rho}$$ where P = sound pressure

for a given sound intensity, P is about 35dB larger in water than in air. The emitted power is therefore larger when transmitted through a more dense medium.

Further, from the equation,

$$\lambda = \frac{c}{f}$$

where λ = wavelength

c = propagation velocity

f = frequency

the increase in c in water means that a given frequency has a wavelength which is approximatly five times that in air. The net result is that, by using a more dense medium for sound transmission, small insects can emit high intensity sounds in the low kHz range (Michelsen 1983).

One of the simplest ways of producing substrate vibration is by the percussion method. This involves the insect (or part of it) applying a force directly to the medium. It is used by many beetles, which knock or drum their heads on the substrate. The rythum of the drumming is species specific (Tschinkel and Doyan 1976).

Land bugs of the family Cydnidae, and Phymatidae (Gogala and Cokl 1983),
use stridulation and tymbal mechanisms similar to those used by larger insects to produce air-borne sounds. The frequencies range from a few Hz up to about 1kHz and are

propagated through the plant stems and leaves on which they sing (Gogala 1985b). Ants use an abdominal stridulatory device to produce vibrations which propagate through the soil and warn other ants when a nest collapses (Masters, Tautz, Fletcher and Markl 1983).

Whirligig beetles (Gyrinidae) produce vibrations at the air/water interface during swimming. These waves are used for echolocation. Using Johnston's organs (specialised receptors at the base of the antennae: Hutchings and Lewis 1983) they detect the waves reflected from an object and so avoid it (Tucker 1969).

The advantages accruing from the use of substrate-borne vibrations have not only been exploited by small insects such as bugs and ants. Male bushcrickets stridulate at the tops of plants. During this movement the legs and abdomen produce vibrations which are propagated through the stems and branches (Keuper and Kuhne 1983). These vibrations may be used at short range by receiving individuals to enhance their localisation of the sound source. In behavioural experiments (Latimer and Schatral 1983), bushcrickets presented with a choice between airborne sound only and sound and vibration, invariably chose the later. Also, some of the auditory neurones in the ventral nerve cord show better coding of the species song when this is presented simultaneously with vibratory signals (Kalmring, Kuhne and Lewis 1983).

Although in many cases communication is limited to

short range courtship songs, the effectivness of substrate-borne transmission means that many land bug species can communicate with male calling and aggression songs. The information carried by these vibrations can be just as specialised as those transmitted by air-borne sounds. Further, since substrate-borne vibrations are often a concommittant of airborne sound production it is difficult, and indeed wrong, to consider only airborne stimuli when discussing insect sound.

There are however, in terms of signal integrity, some disadvantages in using vibratory signals. They have highly complex wave forms and broad band frequency spectra (Markl 1983); the different frequencies are propagated at different rates and show marked frequency-dependant attenuations; reflections occur at each branching point and at the top and base of the plant because of an impedance change. Thus, the frequency content and temporal structure of the signal at the site of the receiver may not be the same as the emitted signal. Gogala (1985a) has suggested that insects may have exploited even this physical phenomenon. Since some can discriminate between relatively low and high frequency vibrations (Cokl 1983), the different propagation rates may provide information about the distance the signal has travelled, thus allowing the receiver to localise the sender.

(ii) Specialised aids for communication.

The most intriguing specialisations for enhancing sound transmission occur in the Gryllidae, where some species modify their environment for acoustic purposes.

Oecanthus burmisteri is a small tree cricket which produces a very pure tone song of 2kHz (Prozesky-Schulze et al. 1975). The wavelength of this song is approximately 170mm whereas the diameter of the sound source is 3.2mm. Acoustic short-circuting would therefore seem an inevitable consequence. In addition tree crickets have no specialised harp instead the whole wing acts as a variable resonator tuned to a range of frequencies (Sismondo 1979). Yet the song is very intense. O. burmisteri achieves this by producing a self-made baffle. The cricket bites a hole in a leaf, exactly the dimensions of the tegmina, and sit in it during singing bouts. The effective baffle dimension (front to back distance) is therefore increased from that of the wings alone to that of the wings plus the remainder of the leaf. Thus, the cricket is able to emit a low frequency sound which is propagated far more efficiently in the broad-leaved, dense undergrowth than high frequencies would be.

Mole crickets have also increased the efficiency of their sound production. Gryllotalpa vineae builds a singing burrow in the shape of a double mouthed horn. The cross-sectional area of the horn flares exponentially from the throat to the mouth. The cricket sits in the burrow at

the junction of the throat of the horn and a cavity called the bulb. The wings point backwards towards the mouth. The exponential horn acts as an acoustic transformer, increasing the air load on the wings, so allowing more of the energy to be radiated as sound. The carrier frequency of the song is 3.4kHz and measurements taken 1m above the burrow show that the sound pressure level can reach 92dB and the song can be heard up to 600m away (Bennet-Clark 1970, 1975).

1.1.2. Sound Localisation.

Successful communication will involve the interaction between, as well as the recognition of, partners. There is therefore strong pressure to develop structures and methods for accurately locating a conspecific sound source. Two parameters of the sound wave may be used to obtain information about the spatial position of the sound source. Sound waves may be measured in terms of the displacement of the component particles of the medium, or as a variation in sound pressure. Particle displacement is a vector quantity and is the major component of the sound wave in the near field. A near field receiver is therefore inherently directional. In the far field, pressure is the major component. Pressure however, is non-directional (scalar) so directional information must be obtained by comparing the responses of the two ears separated in space (Lewis 1983).

Medium displacement is used by many insects for short-range communication. One example of the use of this parameter is in the courtship of Drosophila melanogaster. Similar conclusions have been reached concerning the responses of the Johnston's organs at the base of the antennae of male mosquitoes. In contrast to the antennal flagellae of the female, the antennae of the male are plumose with whorles of long fibrillae (Hutchings and Lewis 1983). The maximum sensitivity of the males' receptors correspond to the flight tone of a virgin female. Mated females and males produce flight tones nearly an octave higher than the virgins, well outside the range of best sensitivity of both males and females (Belton 1974).

In the far field, using two ears, two cues are available for extraction from the propagated sound wave: time differences and pressure differences between each ear. The speed of sound propagation and the size of insects means that time differences are unlikely to be used. They must therefore rely on the differences in the sound pressure at each ear.

Bushcrickets have exploited the principle that sound will be diffracted by a solid body placed in its path. However, to obtain reasonable differences between two receptors, one facing towards and one away from a sound source, the body must be large, at least 1/10, of the wavelength of the sound. Bushcrickets have achieved this because they produce high or ultrasonic frequencies. Their

ears are situated on the fore tibiae, well away from the body, in regions of minimal diffraction. However, each ear drum (or tympanic membrane) is associated with an air space (or trachea) which passes up the leg to open at a large, acoustic spiracle on the prothorax (Fig 1.2(iii)). Boyd and Lewis (1982) showed that this spiracle is the main port of entry for sound. The horn shape of the trachea amplifies frequencies above about 8kHz by 10-30dB (Hill and Oldfield 1981) resulting in a large sound pressure on the internal surface of the ear drum facing the sound source. At high frequencies the region of the contralateral spiracle (and therefore the ear) will experience minimal sound because of diffraction by the body. Inter-aural intensity differences are therefore high at high frequencies. (Fig. 1.2,(iv)). Moths also rely on pressure diffraction effects when listening for the ultrasonic cries of bats. In this case however, sound entry is conventional, the effect being exerted on the external surface of the ear drum. (Michelsen 1983)

The crickets, on the other hand, have had to overcome the considerable problem of wavelengths which are large compared to their body size. Diffraction by the body at the carrier frequency is only about 3dB - far to little for accurate localisation. In the auditory nerve however, left/right differences of 20-25dB can be recorded (Fig. 1.2(ii)). This is achieved by the use of a pressure difference type of receiver. The ears in the fore-tibiae

Fig. 1.2.

The acoustic tracheal systems of,

(i) the cricket,

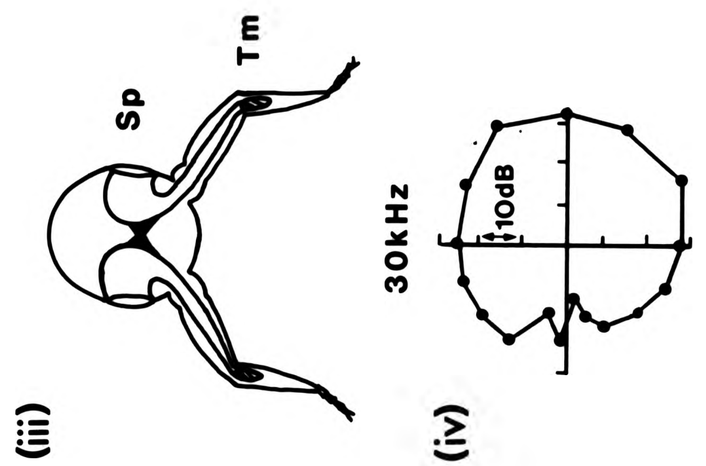
(ii) the bushcricket,

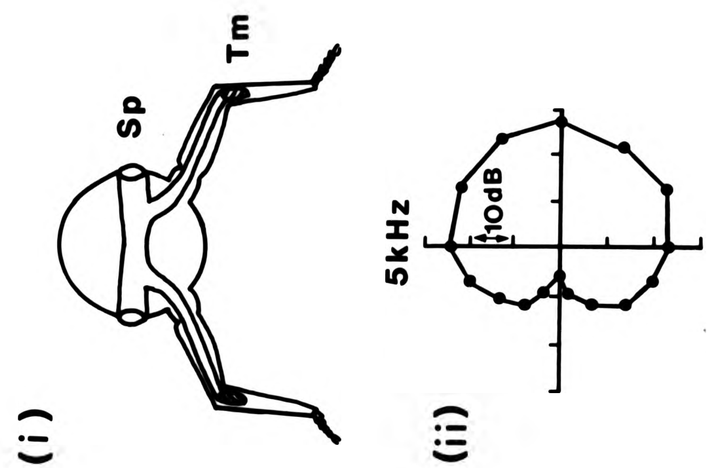
drawn as a transverse section at the level of the prothoracic ganglion, together with a polar plot of the averaged responses of the tympanic nerve, as a function of azimuth of the,

(iii) cricket to a 5kHz tone

(iv) bushcricket to a 30kHz tone

plotted as a sound pressure value calculated by comparing the neural response to an intensity response curve.





are acoustically coupled to each other and to the prothoracic spiracles by tracheal tubes (Fig. 1.2(i)). Since the ear drum is open, sound has access to the external surface; sound also has access to the inner surface *via* the ipsilateral spiracle, the contralateral spiracle and the contralateral ear. This is therefore, a "four input" system in which the net effect is that the response of the ear drum (and therefore the receptor cells) is the result of the difference between the pressure and phase of the sounds acting on its outer and inner surfaces (Lewis 1983)

In acridid grasshoppers the ears function as either pressure or pressure difference receivers, depending on the sound frequency. The ears are located on the first abdominal segment and are connected by a series of air sacs. At low frequencies sound impinges on the external surface of the tympanum, setting it into vibration; it is also transmitted across the body *via* the air sacs to the inner surface of the contralateral ear. This ear will therefore receive sound from inside and outside - a pressure difference system. At higher frequencies however, very little sound is transmitted through the body because of absorption by the tissues. The contralateral ear, at high frequencies is a pressure receiver, sound acting only on the outer surface of the ear drum. Under these conditions left/right differences are obtained by sound diffraction (Miller 1977).

1.1.3. Song Patterns and Species Recognition.

Effective intraspecific acoustic communication requires not only efficient sound production mechanisms but also the recognition of the relevent signal parameters by the receiver. A great number of songs have now been analysed in detail and many behavioural experiments have been performed in an attempt to determine which characteristics of the songs are necessary for species recognition. These approaches have been greatly aided by the development of computer-based sound analysis and by computer controlled methods of song simulation.

In crickets and bushcrickets sound is produced during the closure of the tegmina, the opening being silent. The sound produced during one complete movement of the wings is refered to as a syllable or pulse. Syllables are grouped together in a species specific manner (Fig. 1.3). In crickets, song complexity ranges from the chirps of the courtship (Fig. 1.3(i)a), aggression (Fig. 1.3(i)b), and calling songs (Fig. 1.3(i)c) of Gryllus bimaculatus to the more complex chirps and trills of the courtship (Fig. 1.3(ii)a), aggression (Fig. 1.3(ii)b), and calling songs (Fig. 1.3(ii)c) of Teleogryllus oceanicus (Bentley and Hoy 1972). In bushcrickets the songs are also, generally, resolvable into discrete syllables, although in the more resonant singers such as Rusolia differens the song appears more continuous (Fig. 1.3(iv)). More than one temporal pattern may also occur in bushcricket songs e.g. in

Fig. 1.3.

Diagramatic representations of the temporal patterns of the songs of,

(i) the field cricket, Gryllus bimaculatus

(a) courtship song,

(b) aggression song,

(c) calling song,

(ii) the Australian cricket, Teleogryllus oceanicus

(a) courtship song,

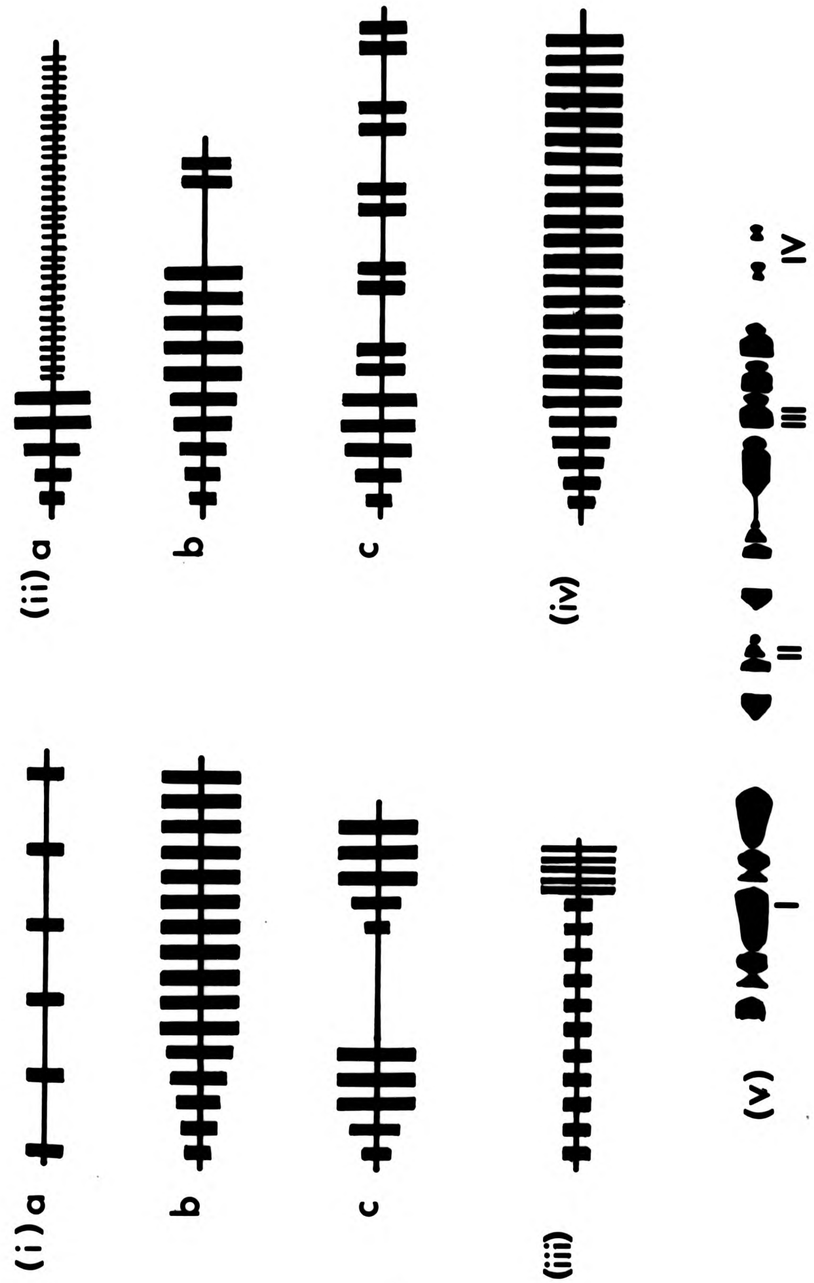
(b) aggression song,

(c) calling song,

(iii) the bushcricket, Platycleis affinis

(iv) the bushcricket, Rusolia differens

(v) the bushcricket, Amblycorypha uhleri



Platycleis affinis (Fig. 1.3(iii)). The most complex song amongst the Ensifera must be that of Amblycorypha uhleri which shows up to four different syllable groupings together with both amplitude and frequency modulation (Fig. 1.3(v); Walker and Dew 1972)

The Ensiferan patterns however, are generally much simpler than those produced by acridid grasshoppers. Since these insects have stridulatory structures on both hind legs, there are two sound sources. Also, sound may be produced continuously during an upward or downward stroke or both. Alternatively, one or both legs may move at different rates within a stroke producing a series of sound "pulses". As if this is not enough, studies by von Helversen and Elsner (1977) using an opto-electric device to record the movement of each leg, showed that the legs do not necessarily move at the same rate: one leg is delayed relative to the other so that, unlike the crickets where syllables are clearly separated by periods of silence, grasshoppers may produce continuous sounds lasting up to 150ms, with no clear structure. It is not surprising then that the terminology to describe these songs is rather confused (Elsner and Popov 1978).

Investigations have been carried out to determine which parameters of the songs are necessary for recognition. Bushcrickets will show phonotaxis in response to their conspecific song (Schatral et al. 1985), and female grasshoppers, if they are virgin, will produce an

agreement song (Helversen and Helversen 1983). In bushcrickets the parametes for recognition are not clear however in grasshoppers, recognition seems to depend on the ratio between the length of the song sequence and the interval between it and the subsequent sequence (von Helversen 1972). In the shorthorned grasshopper (Omocestus viridulus) the tooth impact rate is also important (Skovmand and Pedersen 1978). During courtship in Drosophila melanogaster the male produces low frequency pulses of between 200Hz and 600Hz by flicking the wings. The pulse interval determines whether the female responds appropriately (Bennet-Clark and Ewing 1969). For many vibratory signals, the temporal patterns and frequency modulations are important for species specificity (Gogala 1985b)

As mentioned previously, male crickets produce three song types related to three distinct behavioural stratagies. Recognition of the conspecific song type therefore, forms the basis of cricket behavioural interactions. Spectral analysis of the three song types in T. oceanicus has revealed that, in terms of frequency content, each of the songs are very similar. They each have a carrier frequency at around 4.5-5kHz and harmonics from 10kHz up to 55kHz (Hutchings and Lewis 1984, Latimer and Lewis 1986). Therefore, there can be no basis for song type recognition in terms of frequency in this species. In contrast, in most other species of cricket the courtship

song is produced at a different carrier frequency to the other two songs. In both G. campestris and G. bimaculatus the courtship song has a carrier frequency of 15kHz. In this song the intensity of the 4-5kHz componant is at least 40dB down on the intensity of the carrier frequency (Nocke 1972). The carrier frequencies of the calling and aggression songs are around 4.5kHz.

Since, song type recognition in T. oceanicus cannot be based on frequency differences, the obvious parameter for recognition is the complex temporal pattern of the songs (Fig 1.3(ii); Bentley and Hoy 1972).

There is behavioural evidence that song recognition in T. oceanicus, is based on temporal pattern differences. In some areas of Australia, T. oceanicus exists sympatrically with a related secies, Teleogryllus commodus but the two species remain reproductively isolated (Hill, Loftus-Hills and Gartside 1972). Species isolation is maintained in the field by preferential recognition of the conspecific songs. Comparison between the two species shows differences in the temporal pattern (Bentley and Hoy 1972) and carrier frequency of their calling songs. The carrier frequency of the calling song of T. commodus is between 3.5-3.9kHz whereas T. oceanicus has a carrier frequency of 4.5-5kHz (Loftus-Hills, Littlejohn and Hill 1971). However the frequency difference is not the most important parameter. In behavioural experiments it has been shown that females preferentially track the conspecific

song pattern (Hoy and Paul 1973) even if both are presented at the same frequency (Pollack and Hoy 1979).

Many behavioural experiments have been carried out to investigate which parameters of the conspecific songs of T. oceanicus (Pollack and Hoy 1979; Pollack 1982; Pollack, Huber and Weber 1984; Latimer and Lewis 1986) and of the Gryllus species (Weber, Thorsen and Huber 1981; Thorson, Weber and Huber 1982) are important for recognition. The neurophysiological investigations carried out in this project have concentrated on identification of neurones that may be involved in song recognition in T. oceanicus.

1.2. Neural Processing.

There are three neuronal levels in the auditory sensory pathway of the cricket. Initially, information is received at the tympanal membrane, located on the tibia of each foreleg. The primary afferent fibres in the auditory nerve terminate within the auditory neuropile in the pro-thoracic ganglion where the information is transfered to the interneurones. From the pro-thoracic ganglion the information is carried by higher order neurones to the supra-oesophageal ganglion (brain) where "recognition" is believed to take place.

Single unit recordings from primary auditory fibres of T. oceanicus have shown that they respond to frequencies over the range of 0.5kHz to at least 42kHz, with some units highly tuned to the carrier frequency of the songs

(Hutchings and Lewis 1981). In G. campestris and G. bimaculatus five types of fibres tuned to different frequencies have been identified (Esch, Huber and Wohlers 1980). Of these, groups were found that were tuned to the carrier frequency of the calling song (4-5kHz) and of the courtship song (16kHz). Until recently the morphological basis for the differences in tuning was unclear. However it has now been shown that in crickets (Oldfield, Kleindienst and Huber 1986) and bushcrickets (Oldfield 1982; 1985) the receptor cells are arranged tonotopically with the proximal receptors tuned to lower frequencies than the more distal receptors. By staining the recorded fibres it was shown that those tuned to 4-5kHz project into the pro-thoracic ganglion and terminate within a cresent-shaped auditory neuropile (Esch et al. 1980). The auditory neuropile in bushcrickets is tonotopically arranged (Romer 1983)

In the pro-thoracic ganglion, auditory interneurones with dendritic arborisations within the auditory neuropile, have been identified in both T. oceanicus and the Gryllus species. To date, four anatomical classes of auditory interneurones have been found in the pro-thoracic ganglion: the ascending neurones, in which the axon ascends to the supraoesophageal ganglion; the local, omega neurones which are confined to the pro-thoracic ganglion; the descending neurones which have an axon that projects towards the mesothoracic and abdominal ganglia; and the through neurones which have both an ascending and descending axon.

Because of their ascending axon, which allows information to be carried to the brain, investigations both in this project and by other researchers, have been carried out primarily on the ascending neurones to determine their role in song recognition. In T. oceanicus, two types of ascending neurone have been identified: Ascending neurone A (ANA) and ANB (Hutchings and Lewis 1984). Physiologically, these neurones have best frequencies at around 16kHz and respond to frequencies up to at least 40kHz but show little response to low frequencies in the range of 4-5kHz. In the Gryllus species, two physiological types of ascending neurone have been identified, Ascending neurone 2 (AN2: Wohlers and Huber 1982) which is predominatly, a high frequency neurone and AN1 (Wohlers and Huber 1982) and AN3 (Boyd, Kuhne, Silver and Lewis 1984) which have best frequencies around 5kHz and are therefore low frequency neurones. Ascending neurones tuned to both low and high frequencies have also been identified in another cricket species, Acheta domesticus (Stout, Atkins and Burghardt 1985). Prior to this investigation, no homologous low frequency neurone had been found in T. oceanicus. Since the carrier frequency of all three song types in this species is around 4.5kHz it was expected that a low frequency ascending neurone would be present. This report presents evidence for the existance of a low frequency ascending neurone in T. oceanicus. Its anatomical and physiological characteristics are discussed in relation to its responses

to the conspecific songs.

In T. oceanicus, investigations into the function of ANA have suggested that it is involved in courtship song recognition. When presented with the natural song or simulations of the song, ANA produces an accurately synchronised response to the temporal pattern of the courtship song (Hutchings and Lewis 1984). A role in courtship song recognition has also been suggested for the high frequency ascending neurone in the Gryllus species (Wohlers and Huber 1982). However, this neurone in both G. bimaculatus and G. campestis is tuned to around 16kHz which is close to the carrier frequency of the courtship song, whereas in T. oceanicus ANA is also tuned to 16kHz but the courtship song has a carrier frequency of 4.5kHz. Two-tone experiments have shown that activity in ANA in response to a high frequency tone is actually suppressed in the presence of low frequency tones. Using a computer simulation of the courtship song and the more informative technique of intracellular recording, further investigations into the role of ANA in courtship song recognition were carried out in this project. They confirm the response of ANA to the courtship song, suggest a mechanism by which this occurs and suggest that ANA acts, to to some degree, as a filter for the courtship song.

Investigations carried out on Int-1 (Casaday and Hoy 1977) in T. oceanicus presented strong evidence that this neurone is involved in a predator avoidance behaviour

(Moiseff and Hoy 1983; Nolen and Hoy 1984) similar to that shown by moths in the presence of bats (Roeder 1967). Since crickets fly at night they may also be vulnerable to bat predation. Although there are no direct field observations that T. oceanicus is preyed upon by bats, G. campestris have been observed showing evasive behaviour in the vicinity of hunting bats (Popov and Shuvalov 1977). In the laboratory T. oceanicus, induced to fly in a wind tunnel will show a characteristic avoidance behaviour (negative phonotaxis) in the presence of simulations of ultrasonic bat echolocation signals (Moiseff, Pollack and Hoy 1978). Current manipulation experiments carried out on Int-1 during this flying behaviour have shown that activity in this neurone is both "necessary and sufficent" for the avoidance behaviour to occur (Nolen and Hoy 1984). Despite some differences in the physiological properties of ANA and those reported for Int-1, particularly in response to low frequency sounds (Nolen and Hoy 1986), it is likely that ANA and Int-1 are the same neurone. The apparent conflict between the two functions of this neurone is discussed.

The other group of interneurones in the pro-thoracic ganglion which have been extensively investigated, particularly in the Gryllus species, are the omega neurones. In T. oceanicus, an omega-shaped interneurone called Int-2 (Casaday and Hoy 1977) has been identified. This neurone is anatomically and physiologically similar to the omega neurone of G. campestris (Wohlers and Huber

1978), later called ON1 when a second type of omega neurone, ON2, was discovered. In G. campestris ON1 and ON2 differ anatomically and physiologically. Although both are omega shaped, ON2 has a characteristic dendritic branch which projects across the midline of the ganglion, and is not present in ON1. Physiologically ON1 is a low frequency neurone tuned to 5kHz. Isolated stimulation of each ear has shown that the paired ON1 neurones show mutual inhibition; excitation of one ON1 results in inhibition of the other (Kleindienst, Koch and Wohlers 1981; Wohlers and Huber 1982). In contrast, although the magnitude of the spiking response in ON2 is less than that of ON1, ON2 shows no sign of mutual inhibition (Wohlers and Huber 1982). Prior to this present investigation, no homologue of ON2 had been found in T. oceanicus. This report presents evidence for the existance of a second type of omega neurone in this species which is anatomically similar to ON2 in G. campestris but which shows some striking physiological differences.

Compared to the information available on the ascending and omega neuornes, very little is known about the descending and through neurones. Examples of a through neurone (TN1) and a descending neurone (DN1) have been identified in G. campestris. This report presents preliminary information on a through neurone and two examples of descending neurones in T. oceanicus

The third level of processing of the auditory

information occurs in the brain. Investigations carried out on the brain neurones of the Gryllus species (Boyan 1980; 1981) have led to the identification of specific neurones capable of responding preferentially to conspecific temporal patterns (Schildberger 1984; 1985). Although no recordings were carried out on brain neurones in this project the terminal arborisations of ANA in this ganglion were identified and are discussed in the context of the processing that occurs at this level.

Full understanding of the mechanisms by which the temporal patterns of the different song types are recognised can only be achieved if the connections between the neurones at the different levels can be identified. Until recently connections between neurones were assumed on the basis of comparable anatomical details. However, the use of a dye-killing technique (Miller and Selverston 1979) has now clearly identified connections between the paired ON1 neurones and between the AN2 and ON1 neurones in G. bimaculatus (Selverston 1985). The inhibition shown by ON1 and AN2 as the result of isolated stimulation of the contralateral ear with a low frequency tone was shown to be mediated via the contralateral ON1. Unlike AN2, the inhibition produced in ANA by a low frequency tone is present with free field stimulation. This represents a major difference between the properties of these two neurones. In the experiments described in this report, a series of double electrode recordings, using intracellular

and extracellular electrodes in the same preparation, were carried out to investigate the origin of the inhibition of ANA in T. oceanicus. The results of these experiments and of the dye-killing experiments in G. bimaculatus are compared.

CHAPTER 2.

MATERIALS AND METHODS.

2.1. The Crickets.

Experiments were carried out on adult male and female crickets of the Australian species Teleogryllus oceanicus. The crickets were taken from an established laboratory culture two to four weeks after their final moult. They were kept on a 12 hour light/dark cycle at temperatures of around 25° C with at least 50% relative humidity and fed on a mixture of ground rat pellets and wheatgerm (Bemax). Both sexes were kept together for breeding purposes. The females layed their eggs in petri dishes of moist sand which were removed and replaced every two weeks. The first instars emerged after two to three weeks (Busvine 1955).

2.2. Dissection and Setting-up of the Preparation.

The crickets were first lightly anesthetised using carbon dioxide gas. The wings, antennae and both pairs of hind legs were removed and the insect waxed ventral side uppermost to a perspex platform (15mm by 40mm). The fore legs, on which the ears are situated just below the femero-tibial joint, were waxed at the coxa and tarsi to two right angled pieces of wire fixed to the platform. In this way the leg position resembled the natural posture the cricket adopts while standing. To prevent excessive movement of the ventral cord whilst recording, the gut was removed by first releasing it around the mouth area then drawing it back through a slit in the abdomen. The space

left was then filled with ringer (Fielden 1960) soaked tissue.

The extracellular recordings were carried out in the cervical connectives, between the prothoracic and suboesophageal ganglia. Most of the intracellular recordings were taken from the prothoracic ganglion although in some later experiments, the aim of which was to fill an ascending neuron up to the brain, the axon was penetrated in the connectives between the brain and suboesophageal ganglia. To expose these areas the thin cuticular tissue above them was removed.

During extracellular recordings the area below the connective was packed with ringer soaked tissue to provide support. However for intracellular experiments this preparation proved too unstable for sustained recordings. To overcome the problem of stability, the ganglion was lifted and supported by a silver plated platform (1.2mm wide: Fig. 2.1. SP). In the early single intracellular electrode experiments further support was achieved by waxing the surface of the platform and pinning the ganglion to it by the anterior and posterior connectives. This was however unsuitable for later double electrode experiments which involved an extracellular, suction electrode (Fig. 2.1. SE) recording from one of the anterior connectives. For these experiments stability was achieved by placing a hoop of silver wire (Fig. 2.1. SH) over the top of the ganglion (1.1mm diameter). The ganglion was then held

Fig. 2.1.

Photograph of the experimental set up in the anechoic chamber.

FC - Faraday cage.

IE - Intracellular electrode.

IEM - Intracellular electrode manipulator.

M - Microscope.

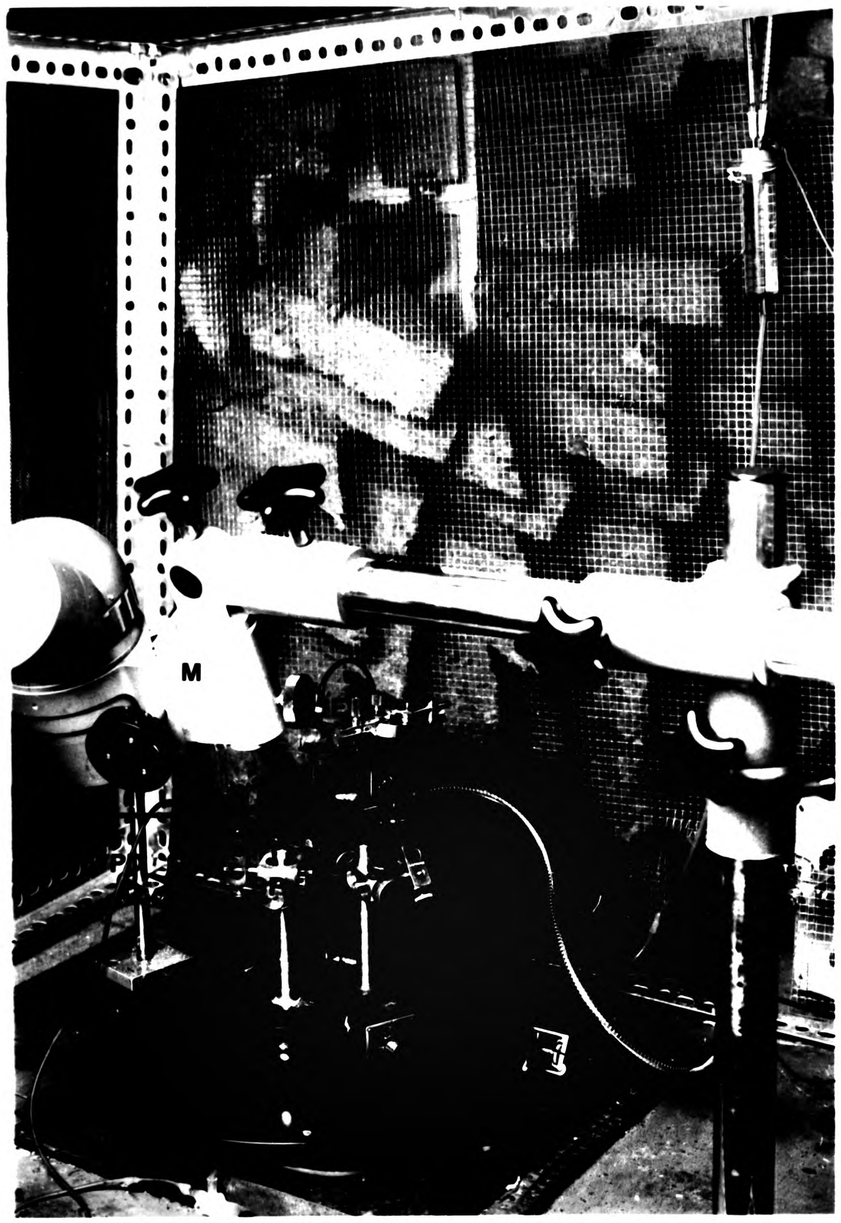
P - Preparation.

PB - Perfusion bath

SE/PD - Suction electrode or Perfusion drip

SH - Silver hoop.

SP - Silver platform.



firmly between the two "spoons" and the electrodes (Fig. 2.1. IE) introduced through the centre of the hoop. With this arrangement the responses from one neurone could be recorded for up to an hour and a half.

The ganglia in the ventral cord of insects are surrounded by a tough membraneous sheath. To facilitate penetration with the electrode, especially the high resistance intracellular electrodes, the ganglion was treated prior to recording with a 1% protease solution (SIGMA Type XIV) for five minutes. The mounted insect was placed in a perfusion bath (Fig. 2.1. PB) and throughout the experiment the preparation was continually perfused (Fig. 2.1. PD) with Fieldens ringer (Fielden 1960), a slightly hypotonic solution which caused the ganglion to swell slightly aiding penetration by the electrode.

2.3. Electrodes.

Glass micro-pipetts were used for both intracellular and extracellular recordings. These were made using 1.2mm glass (Clarkes; GC120F-10) in a standard Scientific and Research Instruments (SRI) puller set to pull electrodes with resistances of between 5-20MΩ for extracellular recordings and between 50-100MΩ for intracellular recordings.

The extracellular electrodes were filled with a 3M solution of cobalt chloride. During recording this solution diffuses passively from the tip and stains the neurone. In

addition, some extracellular recordings were carried out using a suction electrode made from flared plastic tubing, silver/silver chloride wire and a syringe to provide the suction. Although no anatomical detail could result from this method, comparison of the physiological responses with other, previously identified cells, made it possible to identify them. The tips of the intracellular electrodes were filled with a 5% solution of Lucifer Yellow CH (Stewart 1978) in 1M lithium chloride. The barrel of the electrode was filled with 1M lithium chloride. After testing the responses of the impaled cell to the experimental protocol the neurone was stained by injecting the dye using hyperpolarising current.

2.4. Calibration of the Loud Speakers.

The output from the loudspeakers was calibrated from the position of the preparation to determine the sound pressure levels actually received by the cricket. The recordings were carried out in an anechoic room in which the walls were covered with glass fibre wedges to prevent reflection of the sound waves. However the equipment required to obtain stable intracellular recordings may have caused some interference in the sound field. The calibration was measured using a Bruel and Kjaer 1/4" condenser microphone placed, with the diaphram facing the sound source, in the same position as the preparation during an experiment. A continuous pure tone was produced

by a Tone Burst Generator (TBG) and the frequency varied on an oscillator (Farnell, FG2). For each frequency the sound pressure was measured, by a frequency analyser (Bruel and Kjaer: 2107), directly as dBSPL relative to 2×10^{-5} Nm^{-2}, the threshold of human hearing at 1kHz. All dB values given in this thesis are relative to this standard unless otherwise stated. When tabulated these values were used to calculate the sound pressure levels in dBSPL for any given sound level attenuation (Hatfield Attenuator Type 2125).

2.5. Stimulus Generation.

2.5.1. Extracellular experiments.

The stimuli used in these experiments were either single or two tone pulses, 50ms in duration presented at a rate of 2/s. In the two tone experiments the two pulses contained different frequencies and were presented simultaneously.

The frequencies were produced by two oscillators (Farnell), FG2 and FG3 (Fig. 2.2); FG2 produced frequencies between 2 and 200kHz; FG3 produced frequencies from 0.02Hz-22kHz. The frequencies were monitored on a frequency counter (FM: Heathkit: 1m-4100). These sound waves formed the input to two Tone Burst Generators, TBG1 and TBG2 (Taylor 1978) which produced gated, trapeziod pulses with a 5ms rise and fall time, triggered by two channels (S1, S1) of a Grass stimulator (S88). The pulse rate could be altered on the stimulator and the duration of the pulse

Fig 2.2.

Block diagram of the experimental circuitry used for stimulus generation and for recording neural responses during extracellular experiments.

AA - Audio Amplifier.

AC - AC Amplifier.

AC Pre - AC Preamplifier.

At - Attenuators.

CRO1,2 - Cathode Ray Oscilloscopes 1, 2.

E - Electrode.

En - Envelope (stimulus)

F - Filters.

FG2, FG3 - Frequency Generators 2 and 3.

FM - Frequency Monitor.

HFS - High Frequency Speaker.

HS - Head Stage.

LS - Loud Speaker.

LFS - Low Frequency Speaker

PIR - Philips Instrumentation Recorder.

Quad - 2 Channel Power Amplifier.

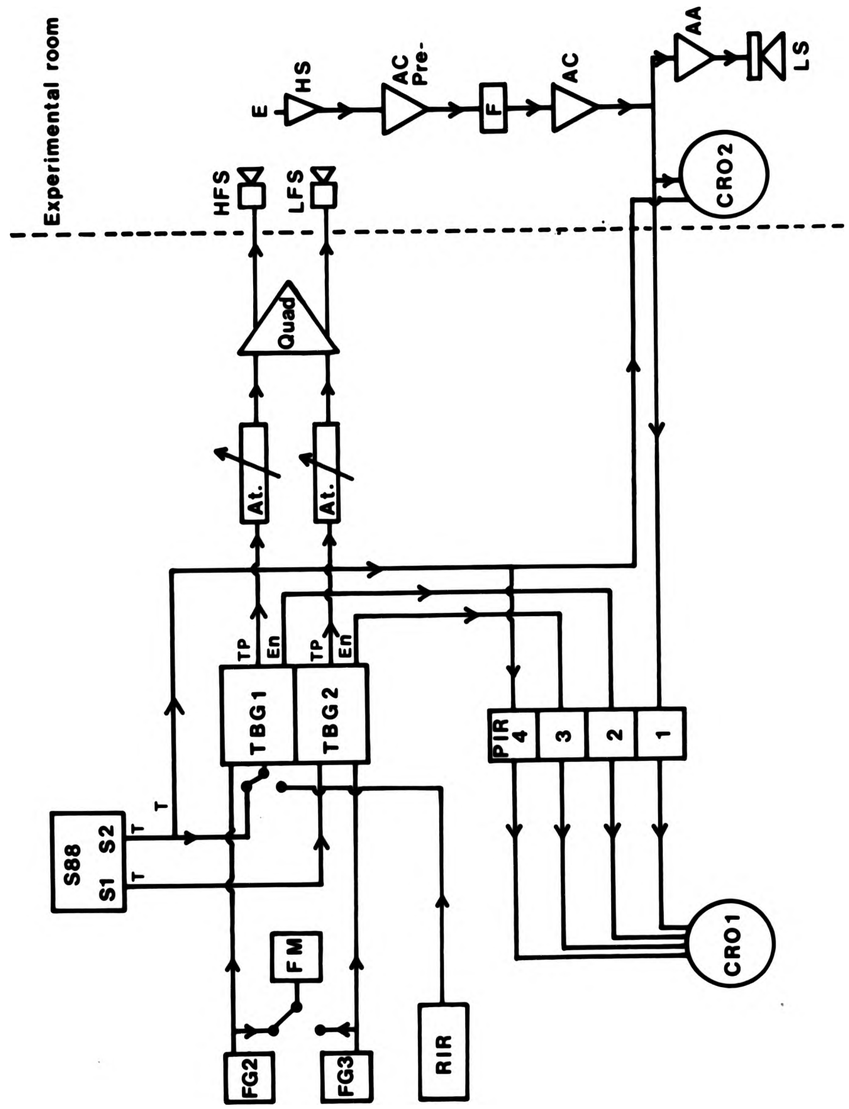
RIR - Racal Instrumentation Recorder.

S88 - Stimulator.
S1: Channel 1
S2: Channel 2.

T - Trigger.

Tp - Trigger pulse.

TBG1,2 - Tone Burst Generators 1 and 2



could be changed between 25ms and 600ms on the TBGs. The output of each TBG was switchable so that either single or two-tone experiments could be carried out. From the TBGs' both signals (Tp) were passed through attenuators (At: Hatfield 2125) in which the signals could be attenuated in 1dB steps over 100dB. The signals were then passed through a power amplifier (QUAD) to the loud speakers: the tone pulse containing the frequency from FG2 passed to the high frequency speaker (HFS), and the tone pulse from FG3 passed to the low frequency speaker (LFS).

Increasing the pulse rate on the Grass stimulator gave a train of pulses which were used to represent parts of the natural songs. However, the temporal patterns of the three song types of Teleogryllus oceanicus are highly complex therefore, it was not possible to produce an accurate simulation of the whole song with this system. A pre-recorded tape (Racal. Store 4: RIR) containing examples of the three natural song types together with simulations of the song patterns containing various frequencies allowed some responses to songs to be obtained. However, the range of frequencies and intensities that could be tested in this way was limited. Since the responses of various auditory neurones to the conspecific songs formed a major part of the intracellular work, a more flexible and accurate representation of the songs was needed. This was achieved by computerising the system and replacing the stimulator with a micro-computer.

The programming for the computer and the building of additional electronic equipment to interface the systems were carried out in collaboration with Dr B.G. Horseman.

2.5.2. Intracellular experiments.

As with the extracellular set-up the frequencies were produced by two voltage controlled oscillators; Farnell function generators (Fig 2.3: FG2, FG3) but during the intracellular experiments the frequency produced by FG2 was controlled by a B.B.C. micro-computer model B (BBC) and second processor (2P). The computer was interfaced to the rest of the equipment by a commercialy available BBC interface, the PCP Interbeeb (I), with an add on digital to analog converter (DAC-PACK: D-A). The output of the D-A formed the input to the VCO via a home built amplifier (A1) which produced a four times gain in the signal to give a range of frequencies, (2-20kHz, 20-200kHz) and also an offset voltage to start the frequencies at 2kHz which was the lowest test frequency used. When addressed (FRED3: ?&FCC3), the frequency entered in the program (TOG) was converted to the appropriate voltage and passed to the VCO which then produced the required frequency (see Appendix for listings of the computer programs).

The computer also triggered the tone pulses from both tone burst generators (TBG1,2). This was done via an 8 bit TTL output port on the Interbeeb. Five of these outputs were used to trigger the external and internal

1

Fig. 2.3.

Block diagram of the experimental circuitry used for stimulus generation and for recording neural responses during intracellular experiments.

AA - Audio Amplifier.

At - Attenuators.

A1,A2 - Amplifiers.

-A - Attenuators.

BBC - BBC Micro computer Model B.

CRO1,2 - Cathode Ray Oscilloscopes 1,2.

D-A - Digital to Analog converter.

DC - DC Amplifier.

DD - Disc Drive.

E - Electrode.

FG2,3 - Frequency generators 2 and 3.

FM - Frequency Monitor.

HFS - High frequency Speakers L and R.

HS - Head Stage.

I - PCP Interbeeb.

LFS - Low Frequency. Speakers, L and R.

LS - Loud Speaker.

M - Monitor.

2P - Second Processor.

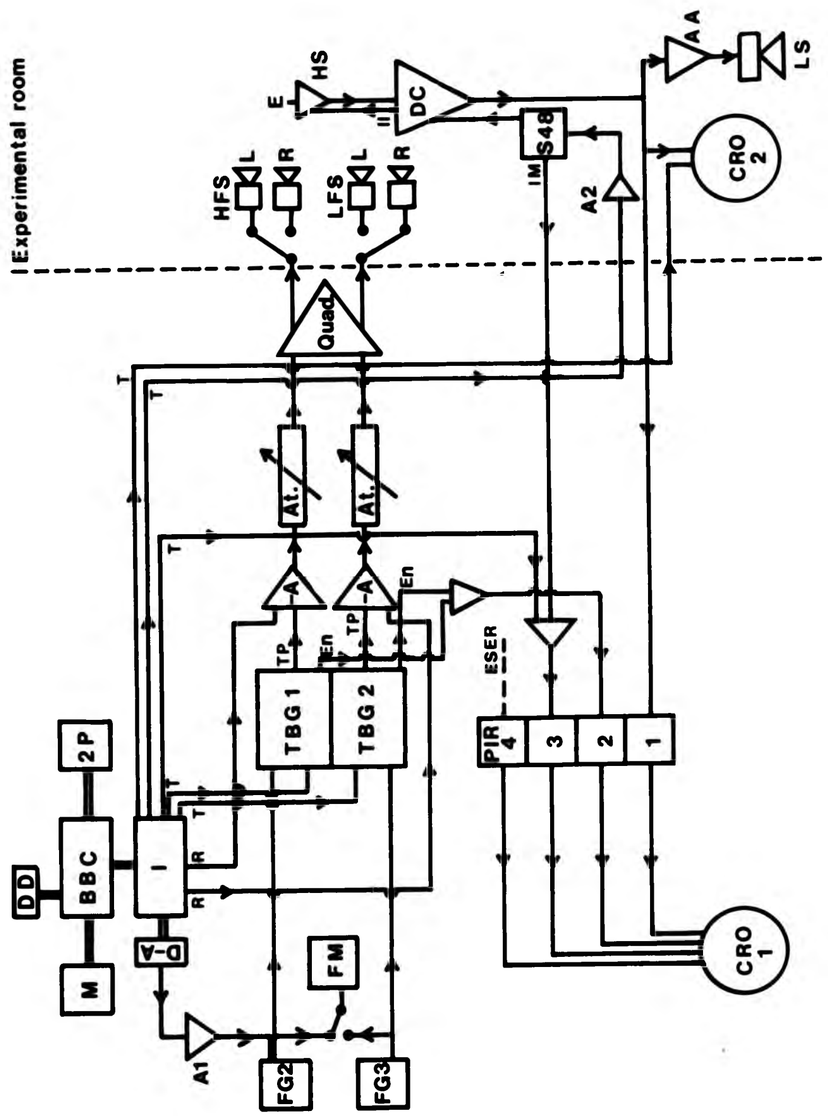
PIR - Philips Instrumentation Recorder.

QUAD - Two channel power amplifier.

S48 - Stimulator.

TBG1,2- Tone Burst Generators 1,2.

En - Envelope, ESER - Extracellular Suction Electrode Recording, II - Current Injection, IM - Current Monitor, R - Relay, T - Trigger, Tp - Tone pulse.



oscilloscopes (CRO1, CRO2), both tone burst generators (TBG1, TBG2) and the single channel Grass stimulator (S48) used to produce current pulses. Each output port has a particular value (n) and when addressed in the program (FRED2: ?&FCC2=n) the appropriate piece of equipment is switched on. By summing the values of the output ports a combination of this equipment can be switched on. Both oscilloscopes can be triggered directly by the TTL pulses produced by the output ports however this was insufficient to trigger the grass stimulator to produce current pulses. Therefore, the signal was first amplified through a home built, non-inverting amplifier (A2).

Apart from computer control of the tone burst generators and FG2, stimulus generation for the intracellular experiments was similar to that for the extracellular experiments. Once triggered both tone pulses were passed through attenuators (At) before being amplified by the two channel power amplifier (QUAD). From here they passed to two sets of high (HFS) and low frequency speakers (LFS) situated opposite each other so the sound could be switched to either the right or left side of the preparation.

Therefore, during these experiments the micro-computer was used to control frequency production from a voltage controlled oscillator and trigger the production of tone and current pulses. Options in the program allowed the frequency of the pulses, the pulse

rate, the relative delays of both the tones and current pulses to be altered each time the program was run. A second program was developed which produced simulations of the three different species songs (A.TOSONG). This program triggered short duration pulses, (15ms), at rates corresponding to the syllable rates of the calling, aggression and courtship songs of T. oceanicus. By activating the relay outputs in the Interbeeb (address: FRED1; ?&FCC1) connected to two home built attenuators (-A) it was possible to reduce the intensity of the trill phase of the simulated courtship song by 6dB to correspond with the amplitude modulations which occur in the natural song. Options in this program allowed the songs to be presented either at a single frequency or with two frequencies found in the natural songs. Each of the different song types could be repeated alone or all three together in a repeated sequence. The program also allowed only the trill phase of the courtship song to be repeated with changes of the pulse rate.

2.6. Response Recording Techniques.

Basically two types of recordings were carried out. The early experiments involved extracellular recordings using cobalt chloride filled glass micro-electrodes. Later experiments involved mainly intracellular recordings using the fluorescent dye Lucifer Yellow CH to mark the cells. In addition, double electrode recordings were carried out with

the simultaneous intracellular recording of one cell together with an extracellular recording, using a suction electrode, of the responses of another cell.

2.6.1. Extracellular recordings.

The glass micro-electrode, positioned in an electrode holder, was attached to a Clarke's hydraulic microdrive to manipuate the electrode into position. The responses of the ascending auditory units where recorded from their axons in the cervical connectives, penetration of which was helped by packing the area below the ganglion and connectives with ringer-soaked tissue to give support. When the electrode was sufficiently near a single auditory unit to give a good signal to noise response to the stimuli the experimental protocol was carried out and the cell stained by passive diffusion of the cobalt chloride. The test stimulus used was generally a 10kHz, 50ms pulse. This frequency proved the most appropriate for picking up both low and high frequency units.

Once a unit was located its response was passed, via a short flying lead attached to the electrode holder, to the headstage (Fig. 2.2: HS: Neurolog: NL100). The indifferent electrode, attached to ground at the headstage, was a piece of silver/silver chloride wire placed in the abdomen. From here the signal was amplified through an AC Pre-amplifier (AC Pre: Neurolog: NL103) then passed through a set of filters (F: Neurolog: NL125) From here the signal

was further amplified (AC Amplifier, Neurolog: NL105) and then passed to an audio amplifier (AA: Neurolog: NL120) to give an auditory representation of the response. The response of the unit was monitored from the AC Amplifier on two oscilloscopes, one outside (CRO1) and one inside (CRO2) the experimental room and recorded, for later analysis, on a Philips Instrumentation Recorder (PIR) at 15 ips. The sound envelopes (E) from both tone burst generators and the stimulus trigger (T) from the Grass stimulator (S88: S2) were recorded on the other three channels.

2.6.2. Intracellular recordings.

For intracellular experiments greater control and accuracy in the manipulation of the electrode was achieved by using a Zeiss sliding micro-manipulator. The glass micro-pipette in the electrode holder was attached to the headstage and the preparation was earthed through the perfusion bath. On penetration of a cell its responses to the auditory stimuli were passed, via the headstage (Fig.2.3: HS) to a DC Preamplifier (built in the laboratory by Dr B.G. Horseman). This amplifier had facilities for capacitor compensation, current injection and electrode resistance measurements. From here the responses were passed to an audio amplifier (A.A: Neurolog: NL120) to give an auditory representation of the cell's responses (LS), and monitored on the internal oscilloscope (CRO2). From the DC Amplifier the signal was passed out of the room to be

recorded on the first channel of a Phillips Instrumentation Recorder (PIR), the output of which passed the recording to the external oscilloscope (CRO1) where it was monitored during the experiment. Because of the restrictions on the number of channels available, both on the tape recorder and the external oscilloscope, the current monitor (IM) from the DC Pre-amplifier and the trigger (T) from the output port were linked, as were both stimulus envelopes (E) from the tone burst generators. These were recorded on channels 2 and 3 respectively and then passed to the external oscilloscope (CRO1) for monitoring during the experiment. Since they were linked the stimulus envelope for a two tone experiment was twice the amplitude of that for a single tone.

2.6.3. Double electrode recordings.

A series of experiments were carried out which involved the simultaneous recording of two different neurones, one extracellularly and one intracellularly. The recording apparatus for each was very similar to those already described except for a few details.

The extracellular recording was made through a suction electrode. This electrode consisted of a syringe attached to a piece of narrowly flared, polythene tubing which when filled with saline formed a connection to a silver/silver chloride electrode. This was in turn attached to the AC headstage and the rest of the extracellular

recording equipment. When the suction electrode was placed over the cut end of a cervical connective and suction applied the tubing formed a high resistance seal around the connective from which the responses could be recorded. The extracellular response (ESER) was recorded on the fourth channel of the tape recorder and monitored on both the internal and external oscilloscopes.

2.7. Identification of Units.

The micro-electrodes used for both the extracellular and intracellular experiments were filled with stains which were used to mark the neurones being recorded. This allowed clear identification of each neurone in terms of neuroanatomical and neurophysiological characteristics.

2.7.1. Cobalt chloride stains.

The electrodes used to record neurones extracellularly were filled with 3M cobalt chloride. During recording this stain leaves the electrode by passive diffusion and marks the neurone. The cobalt ion is relatively slow moving but a contiuous recording of about 30 minutes usualy gave a clear fill showing the input dendritic areas and the axon within the ganglion and cervical connectives.

After recording and staining was complete the prothoracic ganglion along with the mesothoracic and suboesophageal ganglia where dissected out and placed in a

dilute solution of ammonium sulphide for 5 minutes. The tissue was then fixed in ethanol and acetic acid (4:1) for 30 minutes. The ammonium sulphide solution results in the formation of a black precipitate of cobalt sulphide in areas where cobalt chloride is present. If the ganglion was dehydrated and cleared at this stage only the main axon and some of the larger dendritic branches where revealed. In order to reveal the fine dendritic areas the preparations where hydrated and intensified.

The method of intensification used was that described by Davis (1982) based on a modification of the Timms (1958) silver intensification method. The main difference is that with the Timms method and an earlier use of it by Bacon and Altman (1977), the developer used to deposit silver ions on the sulphide precipitate to intensify it was light sensitive. This meant that the process had to be carried out in the dark and so it was difficult to achieve the correct amount of intensification. However this is overcome in Davis' method by using a developer containing tungstic acid which renders the developer insensitive to light. The intensification can therefore be carried out with continual observation. After intensification the preparation was dehydrated and cleared in methyl salycilate and the auditory neurone photographed and drawn using the camera and camera lucida attachments on a Zeiss microscope.

2.7.2. Lucifer Yellow CH Stains.

The tips of the micro-pipettes used for intracellular recordings were filled with a 5% solution of Lucifer Yellow CH in 1M lithium chloride. The rest of the electrode was filled with the lithium chloride. Lucifer Yellow CH is a highly fluorescent dye (Stewart 1978) which can be injected into a neurone using hyperpolarising current. Its main advantage over other fluorescent dyes such as Procion Yellow is its high sensitivity. This means that even a relatively small amount of the dye from a brief injection of current can still result in a faint but complete picture of the neurone allowing accurate identification. This property of the dye proved very useful for some of the less stable units.

Once the test protocol was complete the impaled cell was injected with the fluorescent dye using between 2 and 10nA of hyperpolarising current for as long as the unit was held. The brightest fills were obtained from injections in excess of one hour; however, even 10 minutes gave a sufficiently clear image for identification. At the end of the experiment the suboesophageal, prothoracic and mesothoracic ganglia where dissected out, placed in a fixative of 5% formalin in phosphate buffer at pH7 and left for at least 12 hours. The preparation was then dehydrated in 30%, 50%, 70%, 90% and 100% ethanol and cleared in methyl salycilate. It was then viewed under a Zeiss microscope with a fluorescence attachment and ultra violet

filters, photographed and drawn.

2.8. Picrotoxin Application.

Picrotoxin was used to investigate the subthreshold responses of units recorded intracellularly. Picrotoxin blocks inhibitory synapses by inactivating the chloride ionophore (Cooper, Bloom and Roth 1982). In some of the intracellular experiments, a 5×10^{-7} M solution of Picrotoxin was applied directly to the surface of the ganglion for about ten minutes. The ganglionic sheath was left intact. With this method, the concentration of Picrotoxin at the recording site could not be accurately calculated however the effect of the drug on the response of the neurone could be observed. Direct injection of Picrotoxin through the recording electrode would allow accurate calculation of the dose response curve.

2.9. Replay of Data.

2.9.1. Extracellular experiments.

The results of these experiments were presented in the form of photographs of the analog responses and as dot raster displays. To produce the dot raster displays, the spikes stored on the tape recorder were passed to an AC/DC Amplifier (Neurolog: NL106) then to a spike trigger module (Neurolog: NL200) which discriminates them, producing one TTL pulse for each spike. The +15V pulse from the BRIT output of the spike trigger was used to trigger the Z input

of the oscilloscope to produce a transient brightening of this discriminated spike trace. By adjusting the trace intensity on the oscilloscope each spike was represented by a single dot. Using the storage mode on the scope and a stepping DC offset to move the trace up and down it was possible to show the responses to all eight of the test stimuli at each frequency on the screen at the same time. This was then photographed and spike numbers, latency measurements and any alterations in the responses over 8 presentations of the stimuli could be noted. For each frequency an example of the analog response was taken directly from the tape recorder. The responses to the examples of natural song and of the simulations were also photographed in analog form directly from the oscilloscope screen. Since these were extracellular responses the recording was also passed directly to a second oscilloscope to monitor the signal to noise levels during the dot raster presentation to ensure that each dot represented a spike rather than noise.

2.9.2. Intracellular experiments.

Any subthreshold activity is important with intracellular recordings; thus a dot raster display is unsuitable for analysis of this type of data. Instead the intracellular responses, the stimulus envelopes and the extracellular suction electrode response from the double electrode experiments were taken directly from the tape

recorder and plotted out on paper using a Mingograph (800) chart recorder. Home built stepping attenuators giving attenuations from 1 to 0.01 and gains of between 1 and 5 times allowed the responses to be plotted accurately. The Mingograph provided a continuous record so that any inter-stimulus spontaneous acivity could also be recorded. From these traces spike numbers, latency mesurements and the amplitudes and durations of excitatory and inhibitory post synaptic potentials could be measured.

Some of the data was presented on line as threshold curves. The program (A.TOG) which controlled the triggering of the oscilloscopes and tone burst generators as well as controlling the frequency produced by one of the function generators (FG2) also controlled graphics which allowed the threshold (in dBSPL) of the response to each frequency to be plotted on a graph on the screen. Once the graph was complete it was stored together with information about the location of the electrode and the direction of the sound. At the end of the experiment this curve could be replotted using a replay program (A.REPLAY) which contained options to plot either a single curve or up to four curves superimposed on the same graph.

CHAPTER 3.

RESULTS.

This thesis concentrates mainly on two groups of auditory interneurones: the ascending neurones (AN) and the omega neurones (ON). Additional preliminary data was obtained from a few examples of descending (DN) and through neurones (TN).

Initial experiments involved extracellular, single unit, recordings from axons of the ascending neurones in the cervical connectives. Subsequent, intracellular, recordings provided additional anatomical and physiological information on the ascending neurones and on the omega neurones. In addition, double electrode experiments, involving a simultaneous extracellular recording from an ascending neurone together with an intracellular recording from an omega neurone, were carried out in order to investigate the mechanisms of information processing in the auditory pathway.

3.1. Ascending Neurones.

Two types of ascending auditory neurones were recorded using extracellular micro-electrodes. Identification was based on both anatomical and physiological criteria. The neurones were classified as Ascending Neurone A, which has previously been identified (Hutchings and Lewis 1984) and Ascending Neurone C which was recorded for the first time in this preparation. The third member of this group, Ascending Neuron B (Hutchings and Lewis 1984), was not recorded in any experiment

3.1.1. Ascending Neurone C.

Ascending Neurone C (ANC) was recorded extracellularly in nine preparations using glass micro-electrodes filled with 3M cobalt chloride. Cobalt chloride stains the neurone passively while it is being recorded (Rehbein, Kalmring and Romer 1974). In these experiments the recording time had to exceed 15 minutes in order to produce a stain of sufficient intensity and distribution to allow anatomical indentification of the neurone. Therefore in preparations where the recording time was shorter it was not possible to obtain both detailed neuroanatomical and neurophysiologial information. However by comparing the information from one preparation in which only physiological data was obtained, with one showing both a clearly stained neurone and physiological responses to the test protocol, it was possible to nominate the neurone as being of this type. Of the nine preparations in which ANC was recorded, complete morphological detail was shown in three: in one other preparation the stain was incomplete but was clearly identifiable as ANC. Threshold curves of the excitatory responses of ANC were recorded in all four of these preparations. When compared with other threshold curves the neurone was identified in three more cases. Experiments involving the simultaneous presentation of two tones, containing different frequencies, were carried out on two examples of the neurone. The response of ANC to a pre-recorded tape containing examples of the three

natural songs and simulations of these songs containing different frequencies, were recorded in one preparation, identified as ANC by its threshold curve. In two other preparations only the responses to the song tape were recorded.

3.1.1.1. Anatomy.

The morphology of Ascending neurone C, in the prothoracic ganglion, was revealed by intensifying the cobalt stain (see page 67; section 2). ANC exists as a paired neurone although both left and right neurones were not recorded in the same preparation. An example of one ANC is shown in Fig. 3.1. The axon is situated approximately 40μm from the medial edge of the cervical connective. From here it ascends towards the suboesophageal ganglion but, due to the slow movement of the cobalt ion in relation to the recording time, the axon was never stained beyond half the length of the connective. Situated at the base of the axon, towards the centre of the ganglion, is a dense dendritic area in which all the branches are ipsilateral to the axon. The neurite projets to this dendritic area from the cell body which lies in the contralateral anterior quadrant of the ganglion .

Fig. 3.1

The morphology of Ascending Neurone C (ANC) in the pro-thoracic ganglion. Ventral view.

(i) Photograph of a preparation stained, passively, with cobalt chloride.

(ii) A camera lucida drawing of this preparation. The arrow indicates the anterior direction

Scale bar = 0.1mm

Fig. 3.1

The morphology of Ascending Neurone C (ANC) in the pro-thoracic ganglion. Ventral view.

(i) Photograph of a preparation stained, passively, with cobalt chloride.

(ii) A camera lucida drawing of this preparation. The arrow indicates the anterior direction

Scale bar = 0.1mm

3.1.1.2. Physiology.

Threshold curves.

Extracellular recordings from ANC show it to be most sensitive to low frequency sounds. The threshold levels of response for each frequency were calculated as the intensity necessary to produce an average of one spike over eight presentations of the stimulus. Threshold curves were calculated in seven preparations. The values from these preparations were compared and the maximum and minimum threshold levels for each frequency were plotted as a curve (Fig. 3.2.(i)). ANC shows an excitatory response to frequencies between 3kHz and 20kHz and its best (or characteristic) frequency is around 4-5kHz. In terms of the bandwith of response, the results were consistent between preparations, however, as the shaded area of the curve shows, there are differences in sensitivity at some frequencies. At 4.5kHz the threshold level of the most sensitive unit was 37dBSPL whereas the threshold of the least sensitive unit was 48dBSPL.

Two-tone suppression.

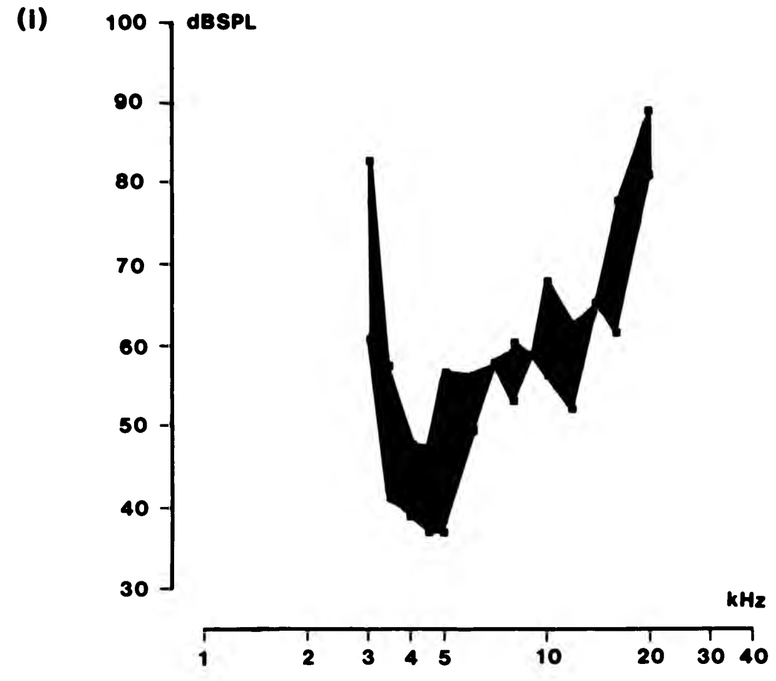
The suprathreshold responses of ANC were investigated using simultaneous presentation of two tone pulses containing different frequencies to determine whether this neurone exibited two tone suppression. Two tone suppression is the term given to a situation in which the excitatory response of a neurone to a single tone

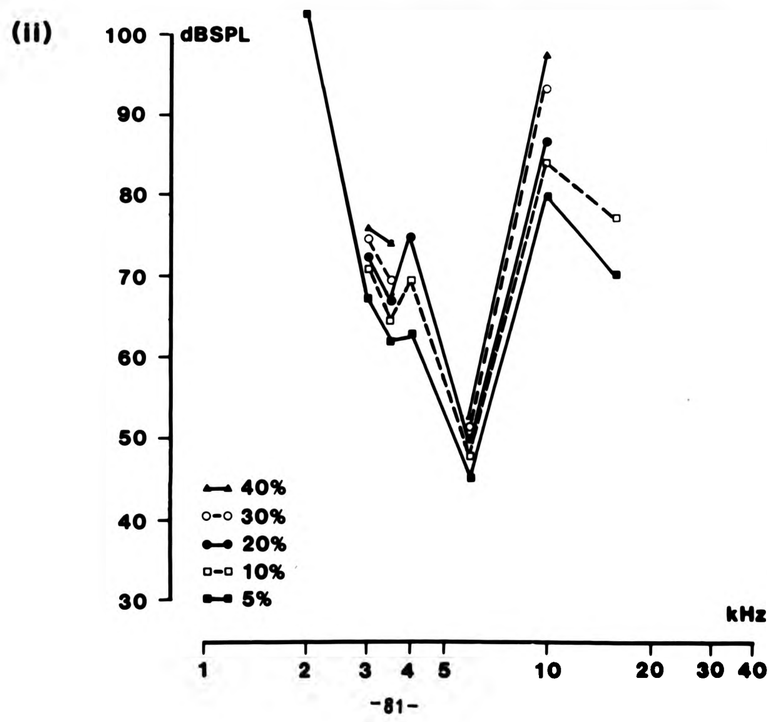
Fig. 3.2.

Threshold responses of ANC.

(i) Composite threshold curve calculated from the threshold values of the excitatory response of ANC in seven preparations. The shaded area represents the maximum and minimum threshold levels obtained for each frequency tested.

(ii) Curves of percentage suppression calculated for ANC showing its response to two tone stimulation. A single control tone of 4.5kHz at 64dBSPL produced an average of 16.5 spikes in ANC over four presentations. The extent to which this response was suppressed by the introduction of a second (test) tone over a range of frequencies and intensities is shown by the curves.





(control tone) is suppressed by the simultaneous presentation of another tone (test tone). ANC has its best frequency around 4.5kHz, so this frequency was used as the control tone. The test tones consisted of frequencies between 2kHz and 16kHz presented over a range of intensities. In response to the single 4.5kHz tone at 64dB, ANC produced an average of 16.5 spikes. This was considered to be the 100% response. Any suppression of this response as a result of the introduction of the test tone was expressed as a percentage decrease of the original response. These values were used to calculate a series of curves of percentage suppression. The threshold of suppression was considered as the intensity of the test frequency necessary to produce a 5% reduction of the original response. The percentage suppression curves calculated from ANC are plotted in Fig. 3.2(ii). The curves show that the best frequency for suppression is around 6kHz with a secondary peak around 3.5kHz. A test tone of 6kHz at 50dB was sufficient to produce a 20% reduction in the response to 4.5kHz at 64dB but it was necessary to present 3.5kHz at 67dB to produce the same result. The maximum amount of suppression produced by any of the test frequencies was 40%.

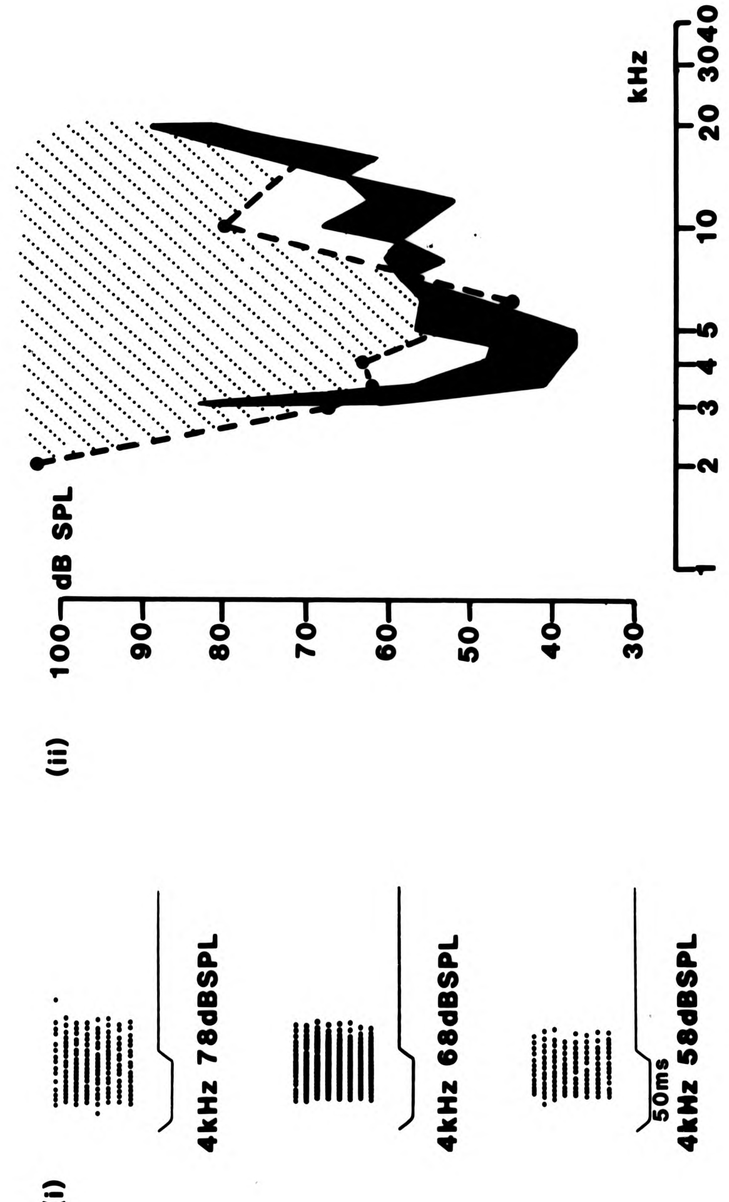
The suppressive effect of some frequencies, shown by presenting them as test tones within a two tone experiment, is significant in terms of shaping the neurone's response to that frequency. This is shown in Fig. 3.3. The ANC

Fig. 3.3.

Physiology of ANC.

(i) Dot raster displays of the responses from a representative example of an ANC neurone to a 4kHz tone presented at 58dB, 68dB and 78dB. Each dot represents one spike and there are eight sequential presentations of the stimulus. Note: ANC shows a reduced response to high intensities of a 4kHz tone.

(ii) The composite threshold curve of ANC (shaded area) plotted together with the curve of 5% suppression of the response of ANC to a control tone of 4.5kHz at 64dBSPL.



threshold curves of excitation and suppression are plotted in Fig.3.3(ii). Part (i) shows dot raster displays of the response of this neurone to a range of intensities of 4kHz: each dot represents one spike and there are eight sequential presentations of the stimuli. 58dB is above the excitatory threshold for 4kHz and produced an average of 13.2 spikes in ANC. At an increased intensity of 68dBSPL, which is well within the excitatory range but only just above the threshold for suppression, the unit showed an increase in response to an average of 20.8 spikes. However at an even higher intensity of 78dB the excitatory response was suppressed resulting in an average of 17.8 spikes.

Song coding.

The responses of ANC were tested, in three preparations, to a pre-recorded tape containing examples of each of the three natural songs. The results are shown in Fig. 3.4. The excitatory response of ANC was correlated, to a varying extent, with the temporal patterns of the syllables in all three of the natural songs. The response to the initial chirp phrases of the calling and courtship songs was clearly correlated with the syllable pattern. However, the intervals between the syllables in the trill phrases of each of these songs were not faithfully reproduced in the responses of ANC. ANC was also presented with a simulation of the temporal pattern of the courtship song containing different frequencies Fig. 3.4(iv). The

Fig. 3.4.

The responses of ANC to the natural songs (NS) and to simulations of the natural songs (SNS).

Upper trace represents the neural response.

Lower trace represents the stimulus.

(i) Responses to the natural,

courtship song - intensity = 68dB

(ii) calling song - intensity = 73dB

(iii) aggression song - intensity = 73dB

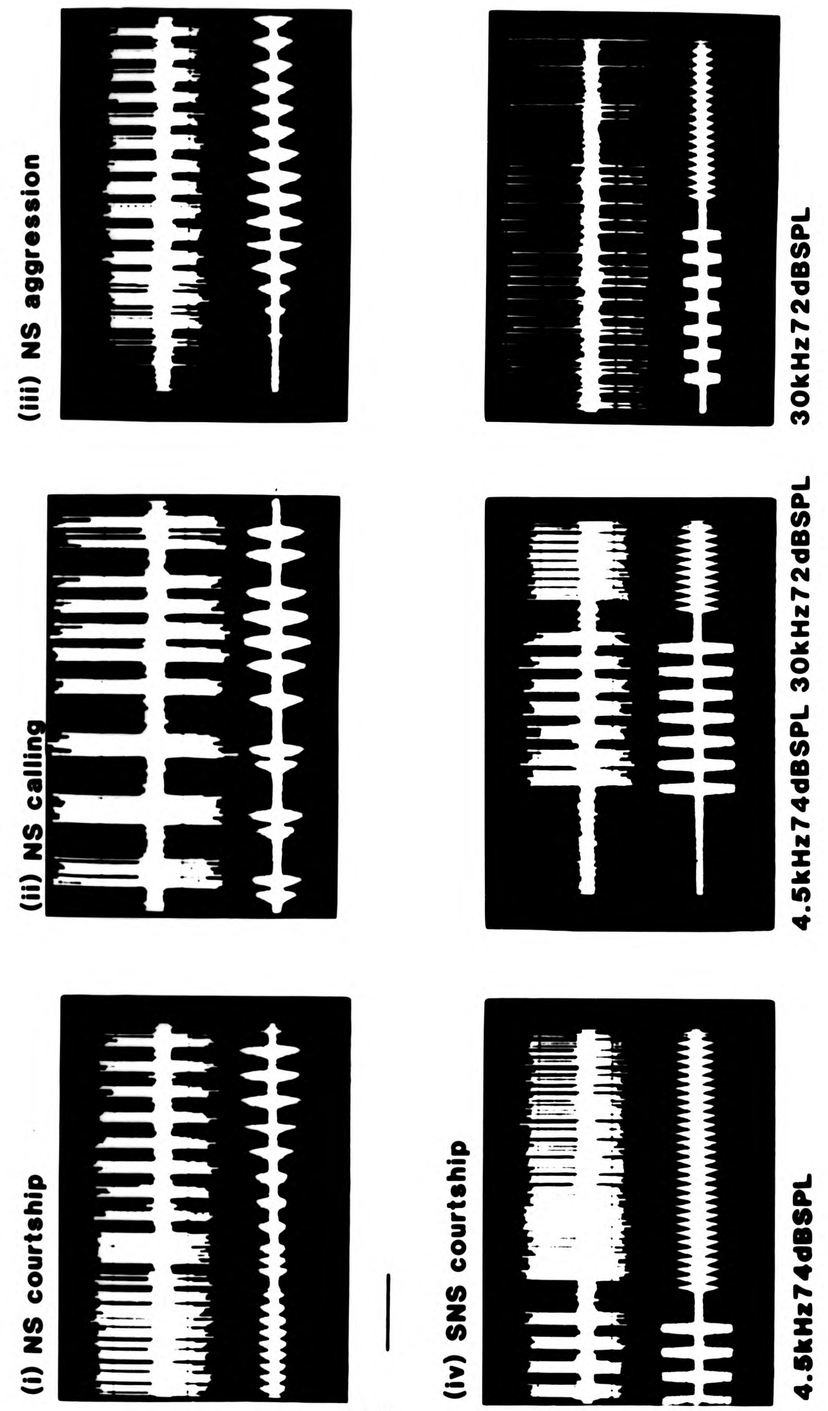
(iv) Responses to simulations of the courtship song containing,

4.5kHz at 74dBSPL,

4.5kHz at 74dBSPL + 30kHz at 72dBSPL,

30kHz at 72dBSPL.

Time scale = 100ms.



simulation containing 4.5kHz at 74dB alone, and the simulation containing 4.5kHz at 74dB plus 30kHz at 72dB produced response in ANC similar to its response to the natural song. However, the simulation containing 30kHz at 72dB alone produced no correlated response at all, the spikes represent spontaneous activity. This was expected because the threshold curve (Fig.3.2(i)) shows that ANC is a low frequency neurone. The frequency spectra of each of the natural songs contains a fundamental at 4.5kHz with, in addition, higher harmonics extending up to 55kHz (Latimer and Lewis 1986). The responses of this unit to the song simulations show that ANC is able to respond to the natural songs because of the low frequency components in their spectra.

3.1.2. Ascending Neurone A.

The responses of Ascending Neurone A (ANA) were recorded using both extracellular and intracellular electrodes. The extracellular recordings were carried out using glass micro-electrodes containing 3M cobalt chloride. During these recordings ANA was passively stained in 18 preparations. The intracellular recordings were carried out using glass micro-electrodes containing the fluorescent dye Lucifer Yellow CH. ANA was stained by injecting the dye into the cell using hyperpolarising current, in 31 preparations. The extracellular recordings provided physiological information on ANA in terms of the spike

response. With the intracellular recordings, information on the neuronal activity at the sub-threshold level was obtained. All the physiological information given in these results is from neurones which have been anatomically identified as ANA or which were recorded extracellularly via a suction electrode.

3.1.2.1. Anatomy.

The morphology of ANA, within the prothoracic ganglion, is shown in Fig. 3.5. In this preparation the neurone was stained by the injection of Lucifer Yellow CH through an intracellular electrode. The axon is situated approximately 40μ m from the midline of the ganglion. Towards the centre of the ganglion it branches to form a dendritic projection which extends out towards the ipsilateral leg nerve. The cell body lies in the anterior quadrant of the ganglion contralateral to the axon and dendritic area. In one preparation the neurone was penetrated in the circum-oesophageal connectives and injected with Lucifer Yellow CH continuously for 2 hours. This produced a stain of ANA over its entire course and is shown in Fig. 3.6. In addition to the dendritic area in the prothoracic ganglion ANA shows extensive arborisation within the supra-oesophageal ganglion. The axon enters this ganglion approximately half way through it dorso-ventrally but once within the posterior protocerebral area it turns sharply towards the dorsal surface. The first main

Fig. 3.5.

The morphology of Ascending Neurone A (ANA) in the prothoracic ganglion. Ventral view.

(i) Photograph of a preparation stained with Lucifer Yellow CH. The dye was injected into the cell, through an intracellular glass micro-electrode, using hyperpolarising current.

(ii) A camera lucida drawing of this preparation. The arrow indicates the anterior direction. Scale bar = 0.1mm.

Fig. 3.5.

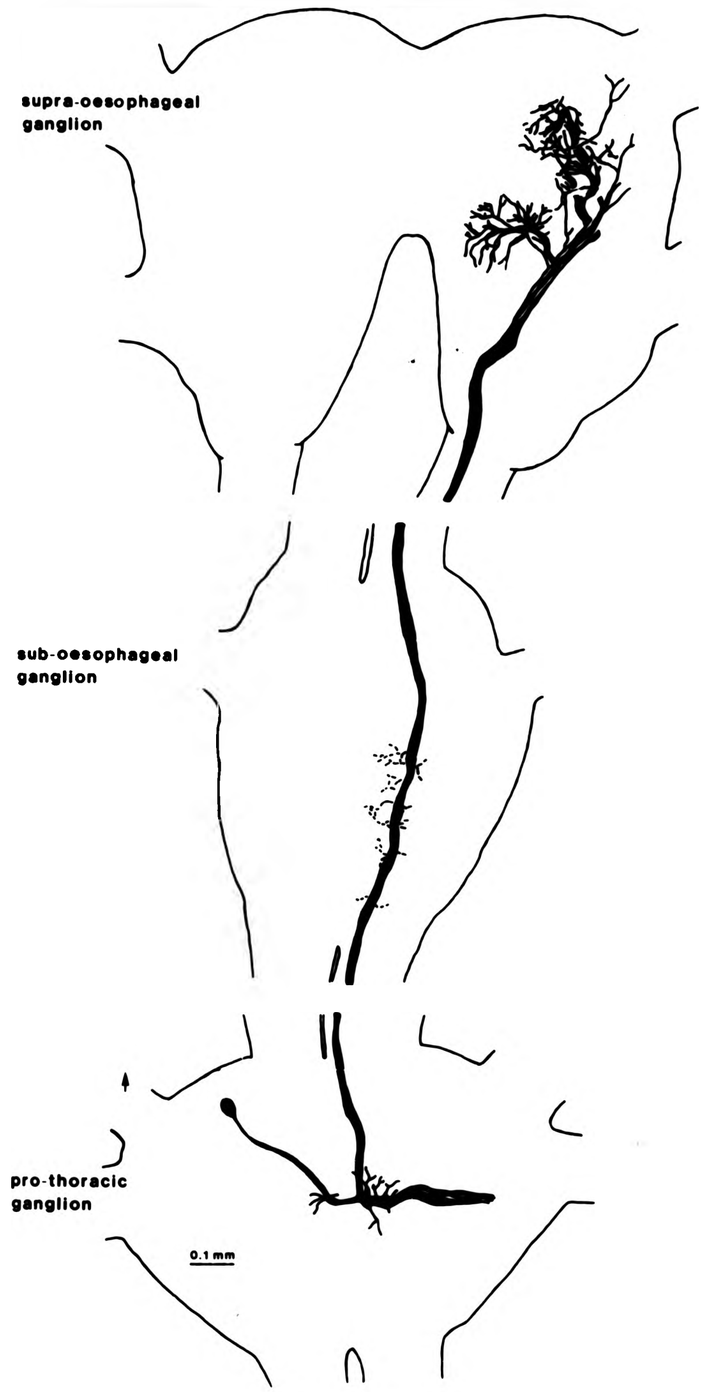
The morphology of Ascending Neurone A (ANA) in the prothoracic ganglion. Ventral view.

(i) Photograph of a preparation stained with Lucifer Yellow CH. The dye was injected into the cell, through an intracellular glass micro-electrode, using hyperpolarising current.

(ii) A camera lucida drawing of this preparation. The arrow indicates the anterior direction. Scale bar = Ø.1mm.

Fig. 3.6.

A camera lucida drawing of a preparation, stained with Lucifer Yellow CH, showing the complete morphology of ANA from the pro-thoracic ganglion, through the sub-oesophageal ganglion, to the supra-oesophageal ganglion. The arrow indicates the anterior direction. Scale bar = 0.1mm.



dendritic arborisation projects ventrally, towards the midline of the posterior protocerebrum; the second main branch moves into the anterior protocerebrum where it also arborises extensively, towards the ventral surface.

3.1.2.2. Physiology.

The physiological information obtained for ANA can be divided into extracellular and intracellular recordings. The extracellular recordings, using either glass micro-electrodes or suction electrodes, provided information on the responses of ANA at the threshold level and above; the intracellular recordings provided additional information on the subthreshold activity underlying these responses.

(a) Extracellular recordings.

Threshold curves.

Physiologically, ANA is a broad band neurone. When tested over a range of frequencies it shows an excitatory response from about 4kHz up to 100kHz. Threshold curves have been calculated for this neurone in 30 preparations. In each preparation the units responded to the same frequency range but showed differences in sensitivity to certain frequencies, in particular, frequencies below 10kHz. In Fig. 3.7 there are four examples of threshold curves recorded from ANA in different preparations to show the range of these variations. Fig. 3.7(i),(ii) represent

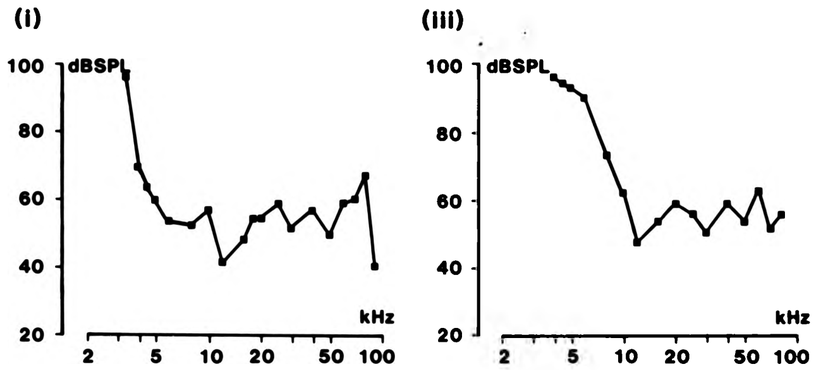
Fig. 3.7.

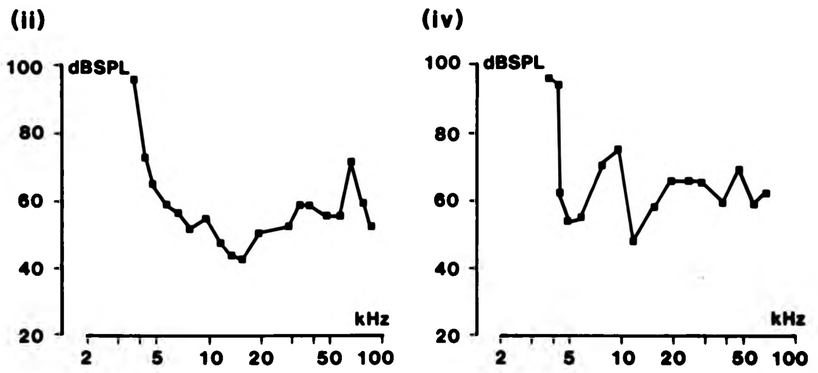
Four examples of threshold curves of ANA.

(i) and (ii) represent the typical response of ANA. A response to frequencies from 3.5kHz up to 100kHz, a best frequency response between 12kHz and 16kHz and a sharp decline in sensitivity to frequencies from 8kHz to 3.5kHz.

(iii) represents an extreme example of the insensitivity shown by ANA to low frequencies. The responses to higher frequencies are more typical.

(iv) shows a second sensitivity peak at 5kHz emphasised by the atypical insensitivity shown in response to 8-10kHz. This preparation did not respond above 70kHz.





the typical threshold curves of ANA. Both curves show an excitatory response from 3.5kHz to 100kHz and a best frequency in the range of 12-16kHz. The neurones are insensitive to frequencies below 10kHz, in particular, to 4kHz and 3.5kHz where they show a sharp roll-off in sensitivity. The unit for which the data is shown in Fig. 3.7(iii) shows the insensitivity at low frequencies to a much greater degree, with the result that there is very little response below 10kHz. The responses to frequencies above this are more typical with a best frequency at 12kHz and an excitatory response up to 100kHz. The threshold curve of the unit shown in Fig. 3.7(iv) is different again. At very low frequencies, below 4kHz, the neurone is typically insensitive; however, at 5kHz it shows a peak of sensitivity with a threshold level of 55dBSPL. This is followed by a sharp decrease in sensitivity particularly at 10kHz where the threshold of response is almost 80dBSPL: in a typical unit the threshold at this frequency is around 60dBSPL. The remainder of the threshold curve shows more typical responses up to 100kHz and a peak of excitation at 12kHz.

Two-tone suppression.

The suprathreshold responses of ANA were investigated with the simultaneous presentation of two tones of different frequencies to test for the occurence of two tone suppression in this neurone. Since ANA shows its

best responses to frequencies between 12kHz and 16kHz the response to a control tone of 16kHz was compared with the responses to the simultaneous presentation of the control tone and test tones in the frequency range from 3kHz to 8kHz. The response to the control tone of 16kHz at 60dB was taken to be the 100% response and any reduction in spike numbers as a result of the introduction of a test tone was calculated as a percentage decrease of the original response. These values were then plotted as a series of percentage suppression curves, which are shown in Fig. 3.8(i). At threshold level (which is taken to be the intensity necessary to produce a 5% suppression of the original response) the peak of suppression occurred at 5kHz. However, at higher intensities of the test tones this best suppression frequency was shifted to 4kHz. In this preparation the 16kHz 60dB control tone produced an average response of 15 spikes. With the simultaneous presentation of a 4kHz tone at 90dBSPL this response was reduced to an average of less than 1 spike over eight presentations. This was not represented on the graph because it was the only frequency to produce this response; it is shown instead as dot raster presentations in Fig. 3.8(ii).

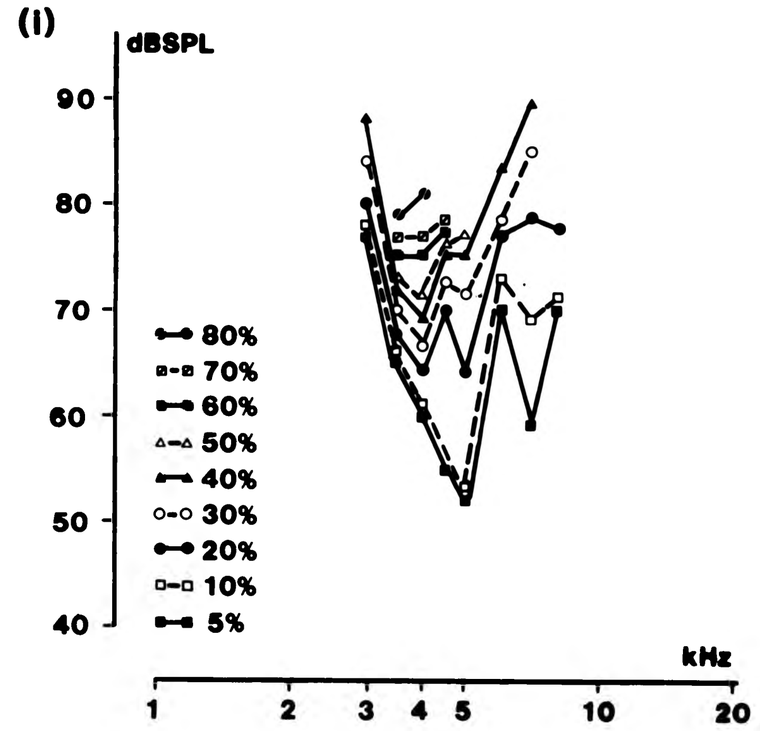
The reduction in spike numbers shown in response to the various frequencies used in the two tone experiments, can also be seen if each of these frequencies is presented alone, over a range of intensities. Fig. 3.9 shows a threshold curve of ANA on which the supra-threshold

Fig. 3.8.

Two tone suppression in ANA.

(i) Curves of percentage suppression of the response of ANA to a control tone - 16kHz 60dBSPL - by the introduction of test tones from 3kHz to 8kHz over a range of intensities. Each point on each curve represents the intensity of that frequency required to decrease the response to the control tone by the percentage stated, e.g 5kHz at 52dB produced a 5% suppression of ANA's response to 16kHz at 60dB.

(ii) Dot raster displays of the response of ANA to eight presentations of the control tone - 16kHz 60dB - alone and together with 4kHz at 90dB.
Note: the test tone reduced the response to the control tone to an average of <1 spike per stimulus.



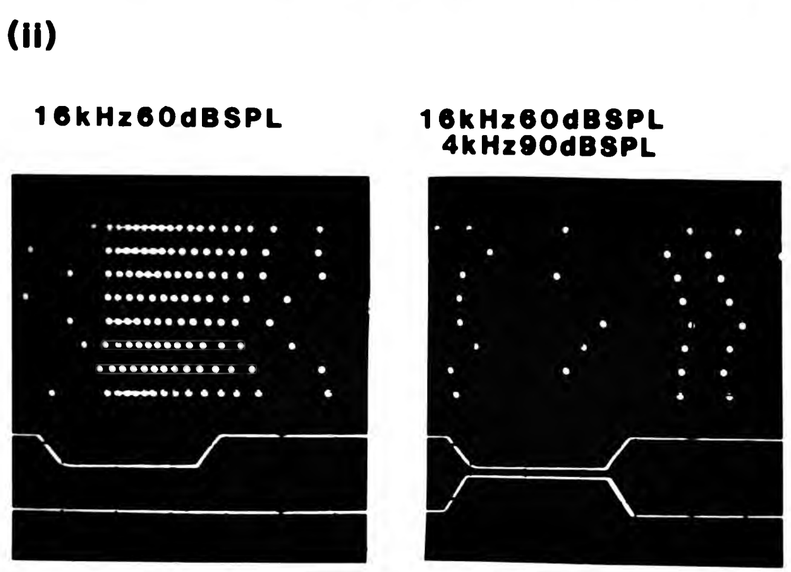
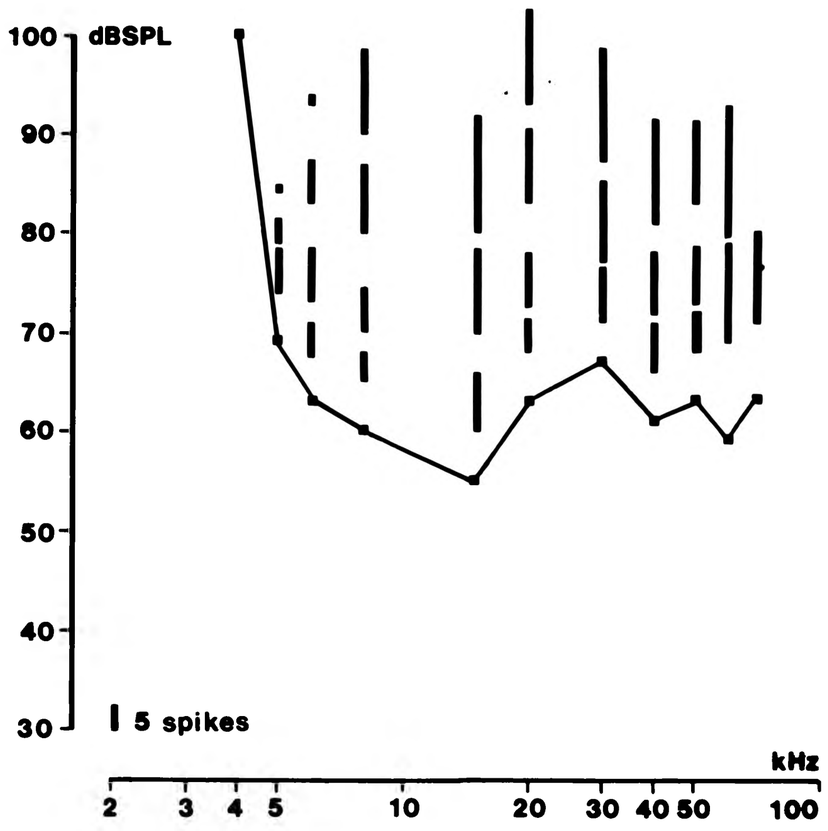


Fig. 3.9.

Threshold curve from one example of ANA plotted together with the supra-threshold response to each frequency over a range of intensities. Each bar represents the number of spikes produced by ANA in response to the frequency at the intensity given by the baseline of the bar, e.g. 5kHz at 74dB, over eight presentations, produced an average of nine spikes in ANA.



responses have been plotted. Each bar on the graph represents the average number of spikes, over eight presentations, shown in response to that frequency at the intensity relative to the base-line of the bar. In this preparation 5kHz at 74dBSPL produced a response of 9 spikes in ANA. At an increased intensity of 79dBSPL this was reduced to 5 spikes. 5kHz at 84dBSPL produced a further reduction in response to 2 spikes. Similarly 6kHz produced a greater response in ANA to low intensites of the tone than to high intensities. Conversely, 16kHz resulted in an increase in response when presented at increasing intensities.

Song coding.

The responses of ANA to the pre-recorded tape of the natural and simulated songs were tested in three preparations. Fig. 3.10 shows that ANA responds with correlated spiking activity to the temporal patterns of all three song types; the courtship song (Fig. 3.10(i)), the calling song (Fig. 3.10(ii)) and the aggression song (Fig. 3.10(iii)). When presented with simulations of the courtship song containing 4.5kHz at 74dB, 4.5kHz at 74dB plus 18kHz at 66dB, and 18kHz at 66dB alone ANA also showed a correlated spiking response (Fig. 3.10(iv)). The previous results showed that the response of ANA is suppressed in the presence of high intensity low frequency tones therefore this correlated activity to courtship song

Fig. 3.10.

Responses of ANA to the natural songs (NS) and to simulations of the natural songs (SNS).

Upper trace represents the neural response.

Lower trace represents the stimulus.

(i) Responses to the natural songs.

courtship song - intensity = 68dB

(ii) calling song - intensity = 73dB

(iii) aggression song - intensity = 73dB

Scale bar = 200ms

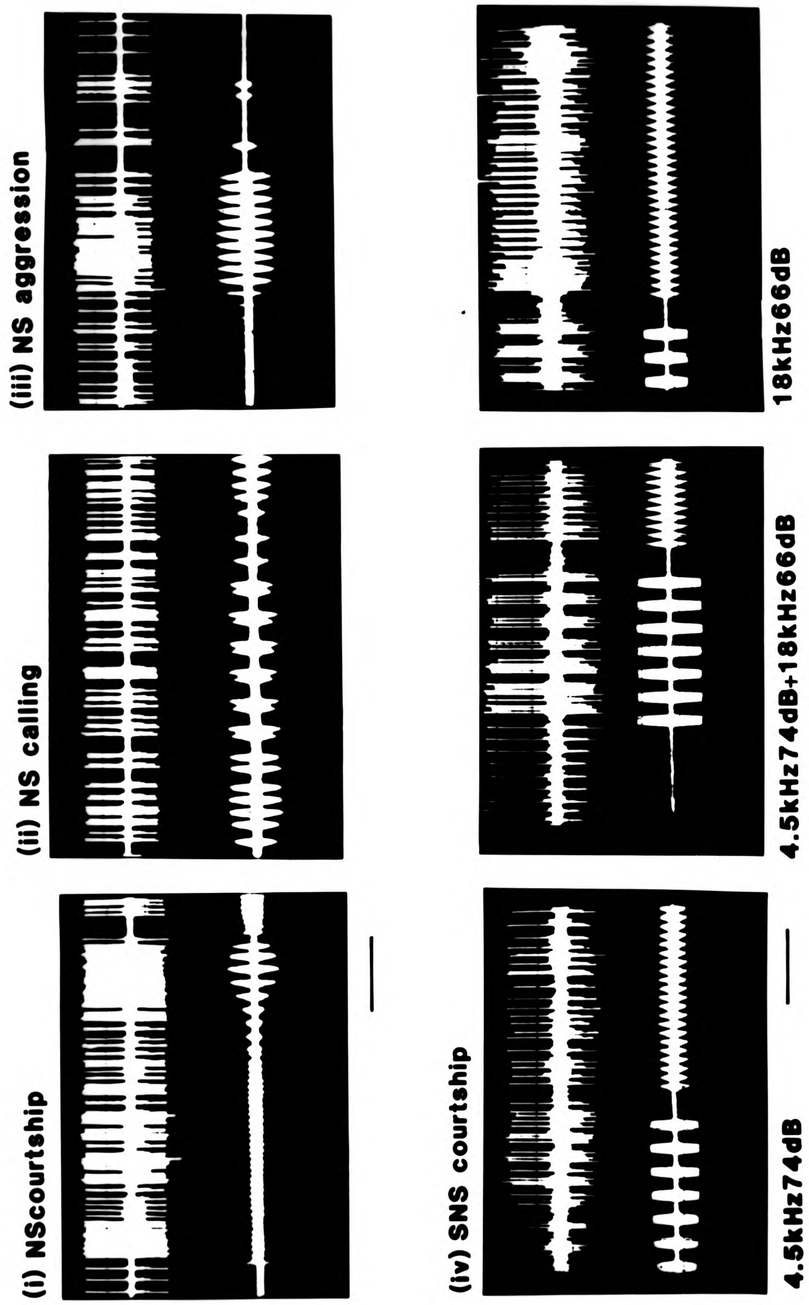
(iv) Responses to simulations of the courtship song containing,

4.5kHz at 74dB,

4.5kHz at 74dB + 18kHz at 66dB,

18kHz at 66db.

Scale bar = 100ms



simulations containing low frequencies was unexpected.

A series of intracellular experiments were therefore carried out to investigate this low frequency response in more detail.

(b) Intracellular recordings.

Using glass micro-electrodes filled with the fluorescent dye Lucifer Yellow CH, ANA was successfully recorded and anatomically identified in 31 preparations. The typical excitatory response of ANA to a high frequency tone is shown in Fig. 3.11(i). In this preparation the recording was obtained from the distal part of the main dendritic area of ANA. As the intensity of the 16kHz tone was increased from 50dB to 80dB the magnitude of the response of ANA also increased. 16kHz at 70dB produced a train of action action potentials: an average of 18 spikes over 4 presentations. Fig. 3.11(ii) shows the neurone's responses in a two tone experiment. When presented simultaneously with both 16kHz (70dB) and a high intensity 4.5kHz tone (89dB) the excitation in response to the 16kHz tone was replaced by a complex inhibitory post synaptic response. Decreasing the intensity of the test tone to 79dBSPL reduced the inhibitory response and the excitatory response to the 16kHz tone at 70dBSPL became evident. Further decreases of intensity to 69dBSPL and 59dBSPL resulted in a predominantly excitatory response. The response to a 4.5kHz tone alone (Fig. 3.11(iii)) shows the

Fig. 3.11.

Intracellular recordings of the response of ANA to,

(i) 16kHz tone presented at 50dB, 60dB, 70dB and 80dB.

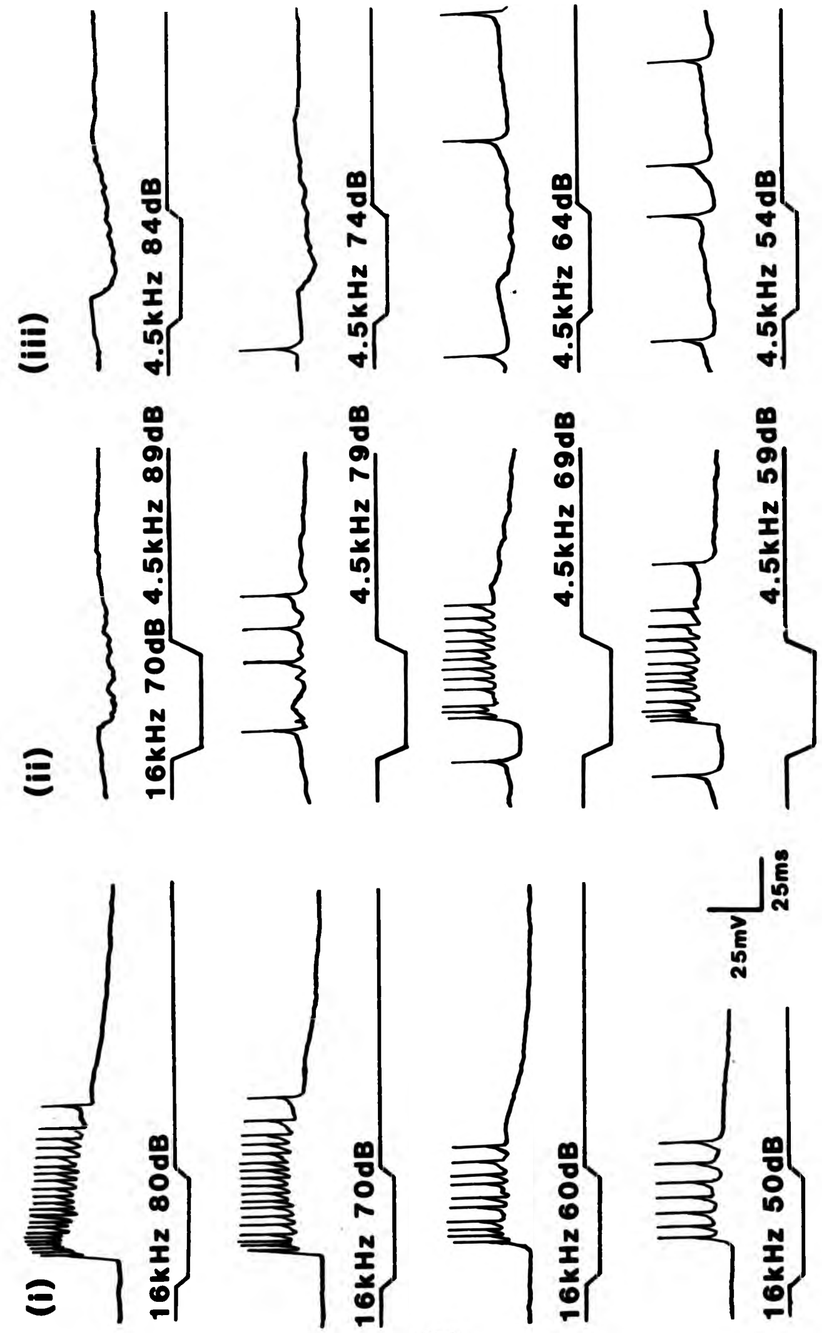
(ii) 16kHz tone at 70dB presented simultaneously with 4.5kHz at 59dB, 69dB, 79dB and 89dB.

(iii) 4.5kHz tone presented at 54dB, 64dB, 74dB and 84dB.

The upper trace represents the neural response.

The deflection of the lower trace represents the stimulus.

Note the inhibition in response to high intensities of the low frequency tone.



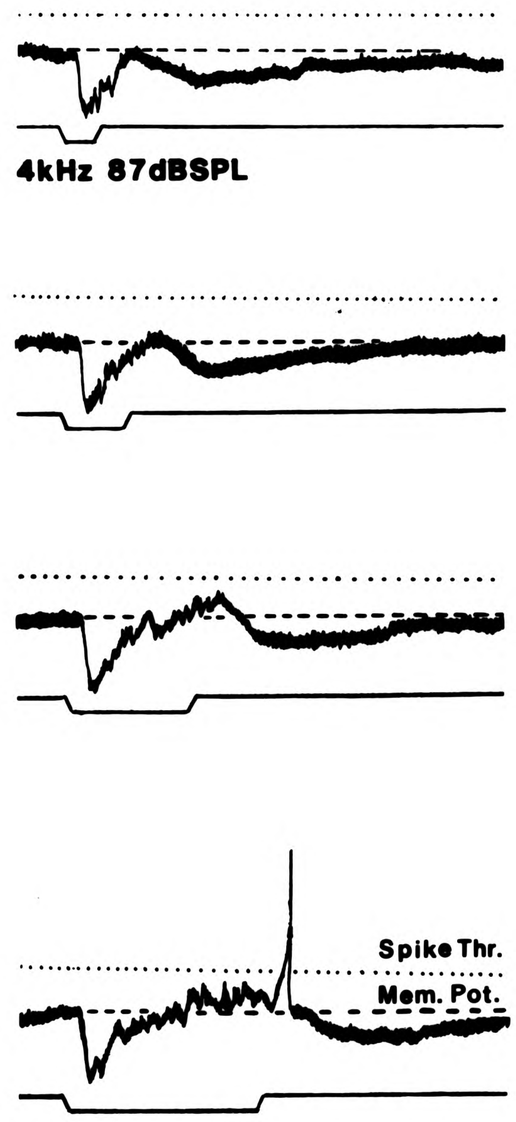
relationship between the intensity of this tone and the amount of inhibition produced. The inhibition was greatest when the tone was presented at 84dBSPL and gradually decreased with decreasing intensity of the tone. At 54dBSPL the neurone's response only slightly interupted the small amount of spontaneous or injury discharge shown by this unit. The inhibitory response to low frequencies is pronounced in this preparation.

Inhibitory and excitatory components of the response of ANA to low frequencies.

When considered in more detail, the inhibition appears to be a complex compound response rather than a single inhibitory post synaptic potential (IPSP). In a preparation in which ANA showed a strong inhibitory response to a short duration stimulus, this response was tested over a range of stimulus durations. Fig. 3.12. shows the responses of this unit to 4kHz 77dBSPL tones of increasing duration. The first stimulus is 25ms in duration and the fourth 160ms. From a resting membrane potential of about -60mV the response to a 25ms duration stimulus consisted of an initial hyperpolarisation which, using the injection of constant current, was estimated in two preparations to reverse at around -70mV. Following this initial response, the membrane potential returned towards resting level. This was then followed by a second, smaller, hyperpolarisation before the membrane potential finally

Fig. 3.12.

Intracellular recordings of the response of ANA to a 4kHz tone at 87dBSPL presented at durations of 25ms, 50ms, 100ms and 160ms. Upper trace represents the neural response. The deflection of the lower trace represents the stimulus. Note that the 160ms duration tone produced a delayed spike response in ANA.



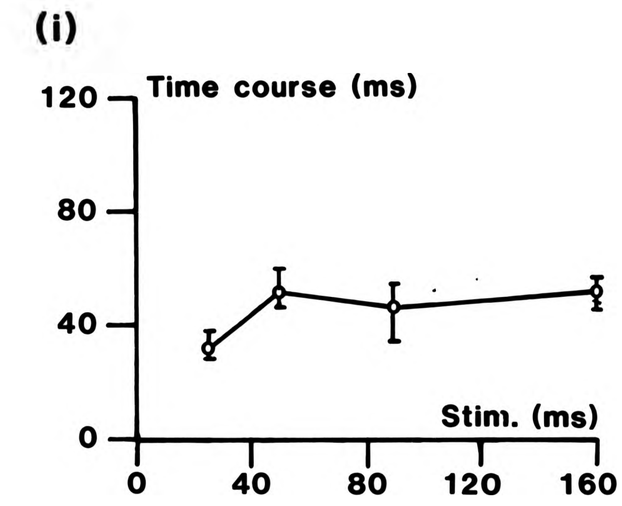
returned to the resting level. As the duration of the stimulus was increased the duration (Fig. 3.12.1(i)) and amplitude (Fig.3.12.1(ii)) of the initial hyperpolarisation remained constant. However, the ampiltude of the following depolarisation increased significantly (Fig. 3.12(ii)). In response to the 160ms stimulus the amplitude of this depolarisation was sufficient to reach spike threshold, resulting in the production of an action potential. The amplitude of the second hyperpolarisation remained at approximately 8mV despite increasing the stimulus duration. The time taken for the membrane potential to return to its original resting level was variable and dependant upon the presence of any subsequent activity in the neurone. Therefore, in this preparation ANA showed complete inhibition to 4kHz at 87dBSPL until the duration of the stimulus was increased to 160ms. The spike which occurs at this duration may be the result of a rebound effect from the inhibition. However, this appears unlikely because the amplitude and duration of the initial hyperpolarisation remains constant in response to each of the stimuli. Therefore, any rebound effect based on the inhibitory response should also have remained constant; yet only the longest duration stimulus resulted in a spike. A more likely explanation is that the spike is the result of an underlying excitatory input which has a longer duration than the inhibitory input. Fig. 3.13 shows the results from another preparation in which even a 50ms duration 4.5kHz

Fig. 3.12.1

The response of ANA to increasing duration of 4kHz at 87dBSPL.

(i) Time course of the initial IPSP phase of the response.

(ii) Amplitude of the hyperpolarising phase and of the depolarising phase of the response.



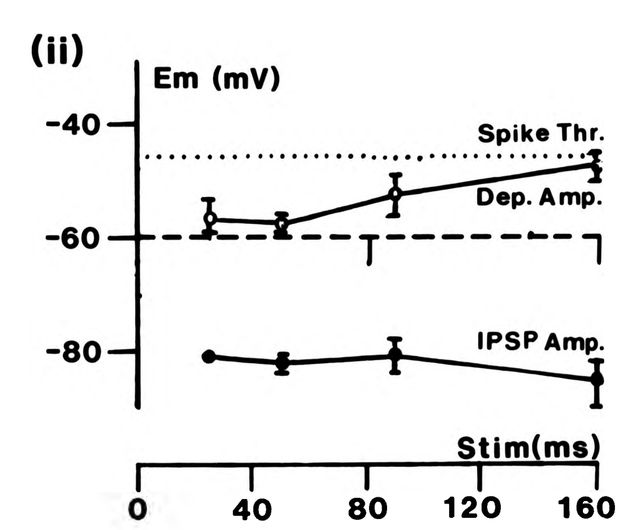
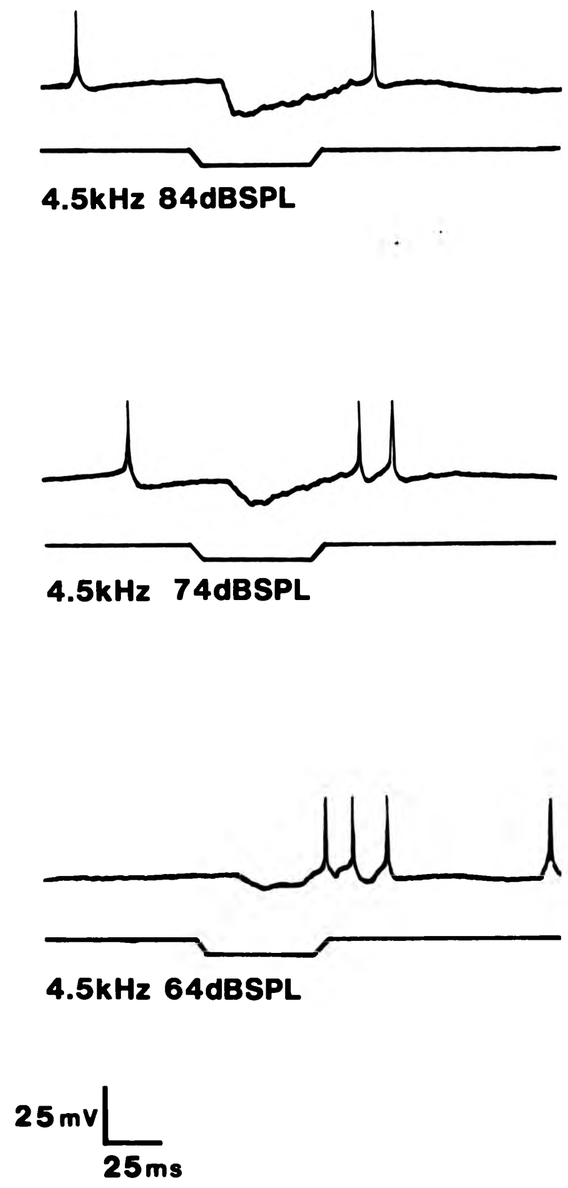


Fig. 3.13.

Intracellular recordings of the response of ANA to a low frequency 4.5kHz tone at 64dB, 74dB and 84dB.

Note the excitation in response to low intensities.



(84dB) stimulus was sufficient to result in a spike. When the intensity of the stimulus was decreased, to 74dB and 64dB, resulting in a reduction in the magnitude of the IPSP, the unit began to respond with increased excitation. The overall response of ANA to low frequencies seems therefore, to be an integration of exitatory and inhibitory inputs.

Effect of Picrotoxin.

To investigate the hypothesis that ANA shows an underlying excitatory response to low frequency sounds which is usually masked by the more prominent inhibitory response, a series of experiments were carried out in which the aim was to block the inhibition and thereby release any underlying excitation. Experiments carried out on the inhibition shown by the T cells in a grasshopper (Suga and Katsuki 1965) suggested that GABA was the neurotransmitter involved in this response. It is therefore possible that GABA is also responsible for mediating the inhibitory response shown by ANA. On binding with its receptor sites GABA produces hyperpolarisation of the post synaptic membrane by increasing its permiability to chloride ions. Picrotoxin is a known antagonist of this effect of GABA and it was therefore used in the experiments in an attempt to block the inhibitory response of ANA. In one preparation the responses of ANA were recorded to a 4.5kHz tone over a range of intensities before and after the application of 5%

10^{-7} M Picrotoxin (Fig. 3.14). Before the picrotoxin was applied, ANA showed hyperpolarisation followed by a single spike in response to 4.5kHz at 84dB (Fig. 3.14(i)). At lower intensities (64dB) the hyperpolarisation decreased and the response consisted mainly of spikes. Ten minutes after the application of Picrotoxin (Fig. 3.14(ii)) the inhibition previously shown in response to the high intensity 4.5kHz (84dB) tone was largely replaced by an exitatory response. As the intensity of the stimulus was decreased to 74dB then to 64dB this excitation increased. Therefore, blocking the inhibitory response with Picrotoxin did reveal the presence of an underlying, longer duration excitatory response. In the untreated preparation most of the excitatory input is suppressed by the inhibitory input but the excitation contributes to the depolarising potential which results in the post inhibitory spike.

To investigate the significance of this response in terms of song recognition ANA was stimulated with simulations of the natural song patterns filled with different frequencies. The extracellular recordings of ANA in Fig. 3.10 show that this neurone can produce a correlated excitatory response when presented with song simulations containg low frequencies. At the intracellular level the effect of the song pattern stimulus can be seen more clearly.

ANA was recorded intracellularly and its responses to simulations of all three song types were determined in

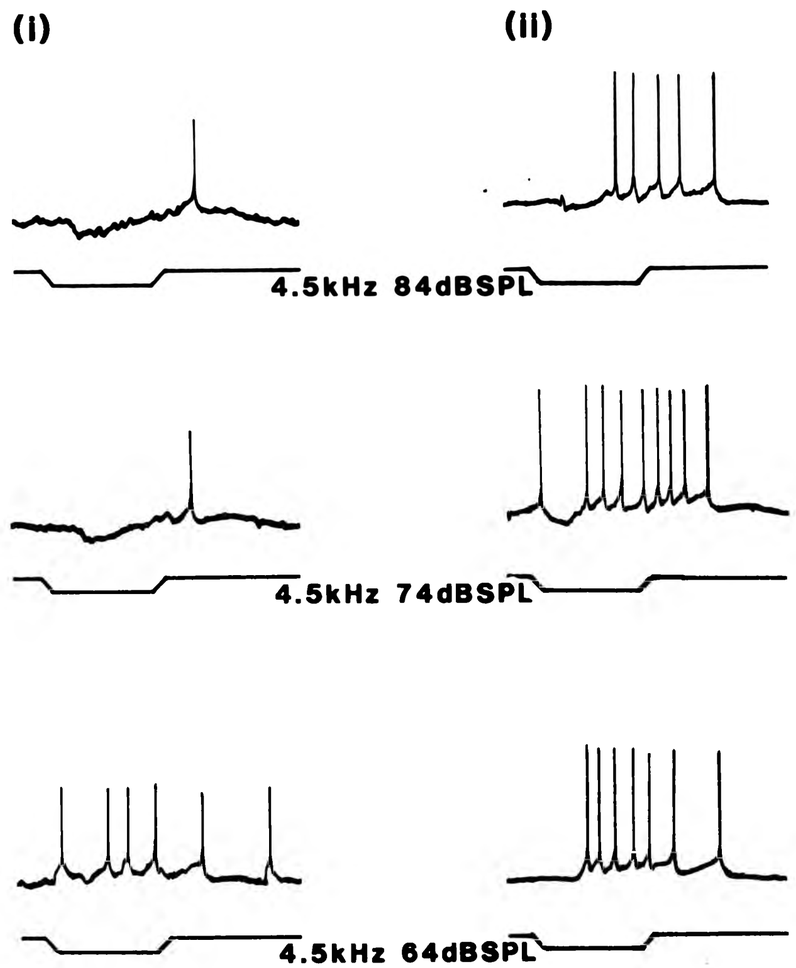
Fig. 3.14.

The effect of Picrotoxin on the response of ANA to a low frequency tone.

Intracellular recordings of the response of ANA to 4.5kHz at 64dB, 74dB and 84dB.

(i) before the application of picrotoxin.

(ii) 10 minutes after the application of Picrotoxin (5×10^{-7}M) directly onto the surface of the ganglion.



seven preparations. The neurone typically produced an accurately correlated response to the trill phrase of the courtship song; both the calling and aggression songs tended to produce a reduced response with less correlation particularly to the doublets of the calling song. Fig. 3.15 shows these results in one preparation. In response to simulations of the three song types containing 4.5kHz at 89dB plus 16kHz at 70dB which represents high intensities of the correct relative intensities of these frequencies in the natural song, ANA produced a correlated response to the trill phase of the courtship song; the calling and aggression songs produced very little response in this neurone. Fig. 3.15.1 shows results obtained from the preparation which produced the responses shown in Fig. 3.12; it was therefore known to produce an inhibitory response to 4kHz at 87dBSPL unless it was presented at a duration of 160ms. This unit was presented with simulations of the courtship, calling and aggression songs containing 4kHz at 87dBSPL. All three songs produced some excitatory response in ANA but the courtship simulation resulted in by far the most accurate response, particularly to the fast trill phrase of this song. In this phrase the spike response was accurately correlated with the temporal pattern in terms of one spike in response to each syllable (Fig.3.15.1(i)). The mechanisms involved in generating this spiking activity to a simulation containing a low frequency sound, are shown in part (ii) of Fig. 3.15.1 in which the response to the

Fig. 3.15.

Intracellular recordings of the response of ANA to simulations of the courtship song, calling song and the aggression song each containing 16kHz at 70dB and 4.5kHz at 89dB.

Note that ANA produced an accurately correlated response to the syllable pattern of the trill phrase of the courtship song.

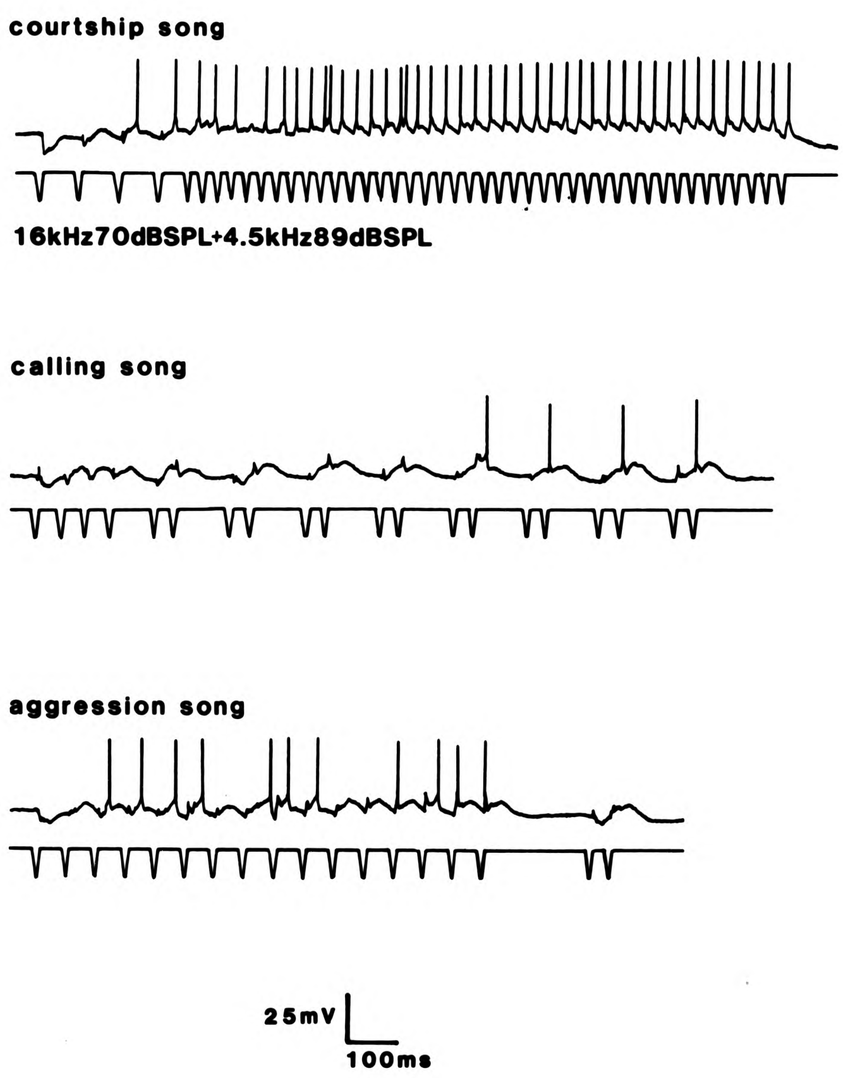
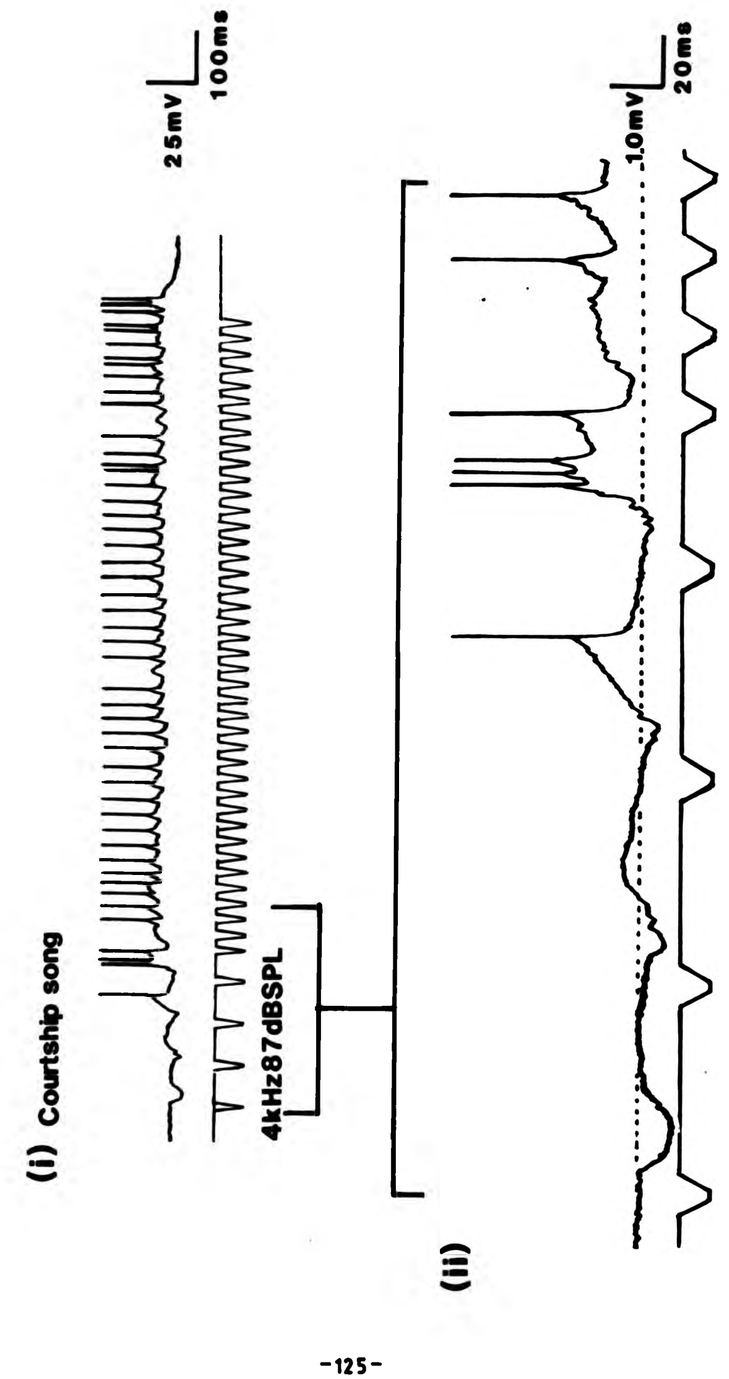


Fig. 3.15.1.

Intracellular recordings of the response of ANA to a simulation of the courtship song containing 4kHz at 87dB.

(i) time base = 100ms

(ii) time base = 20ms



courtship song has been plotted on an extended time base. In response to the first syllable of the song this unit showed the typical inhibitory response consisting of an initial hyperpolarisation followed by a depolarisation. However, the syllable rate of this song is such that the response to the second syllable occurred before the response to the first syllable was complete. Therefore the activity caused by the second syllable was imposed on the compound depolarising potential of the first response rather than on a resting potential. Summation of these responses resulted in the membrane potential being raised so that with the introduction of the response to the third syllable its depolarising potential was sufficient to take it beyond spike threshold and therefore to produce a delayed spike. The fast syllable rate in the subsequent trill phrase ensured that once reached, the membrane potential did not fall far below spike threshold so that each syllable resulted in a spike.

If this interpretation of the response is correct then it would be expected that a reduction in the rate of syllable presentation would result in a reduction in the accuracy of spike coding. This was investigated by presenting the neurone with a simulation of the trill phrase of the courtship song in which it was possible to alter the syllable rate. Four units were tested and each showed comparable responses; the responses from one unit

which showed a strong inhibitory response to low frequency tones are shown in Fig. 3.16. When presented with a simulation of the trill containing 4.5kHz 80dBSPL at a syllable rate of 35/s, which is equivalent to the rate in the natural song, the neurone produced an accurately synchronised response of almost one spike/syllable of the stimulus. A decrease of syllable rate to 25/s also resulted in an accurate response. However, further decreases to 16/s and particularly to 12/s produced an uncorrelated response in this unit.

In addition to the syllable rate the synchronised response in ANA is also dependant on the intensity at which the trill phrase is presented. The response of ANA was tested to a simulation of the trill phrase of the courtship song (35 syllables/s) over a range of intensities of 4.5kHz (Fig. 3.16.1.). At low intensities, near threshold, the response was poor; however from 68dB to 78dB, ANA produced an accurately correlated response. At 80dB the response was less accurate and at 84dB the response showed no apparent synchronisation.

A third component of the song, which affects the temporal coding ability of ANA is the frequency content. More accurate coding can be shown by ANA if the simulation contains a high frequency component to mimic the harmonics in the natural song (Fig. 3.16.2). A simulation of the courtship song containing 4.5kHz at 83dB plus 16kHz at 64dB (the correct relative intensities of these frequencies in

Fig. 3.16.

The effect of syllable rate on the response of ANA to a simulation of the trill phrase of the courtship song, containing 4.5kHz at 80dBSPL.

The syllable rates tested were,

35/s

25/s

16/s

12/s

Note: A rate of 35 syllables/s represents the natural syllable rate in the trill phrase of the courtship song.

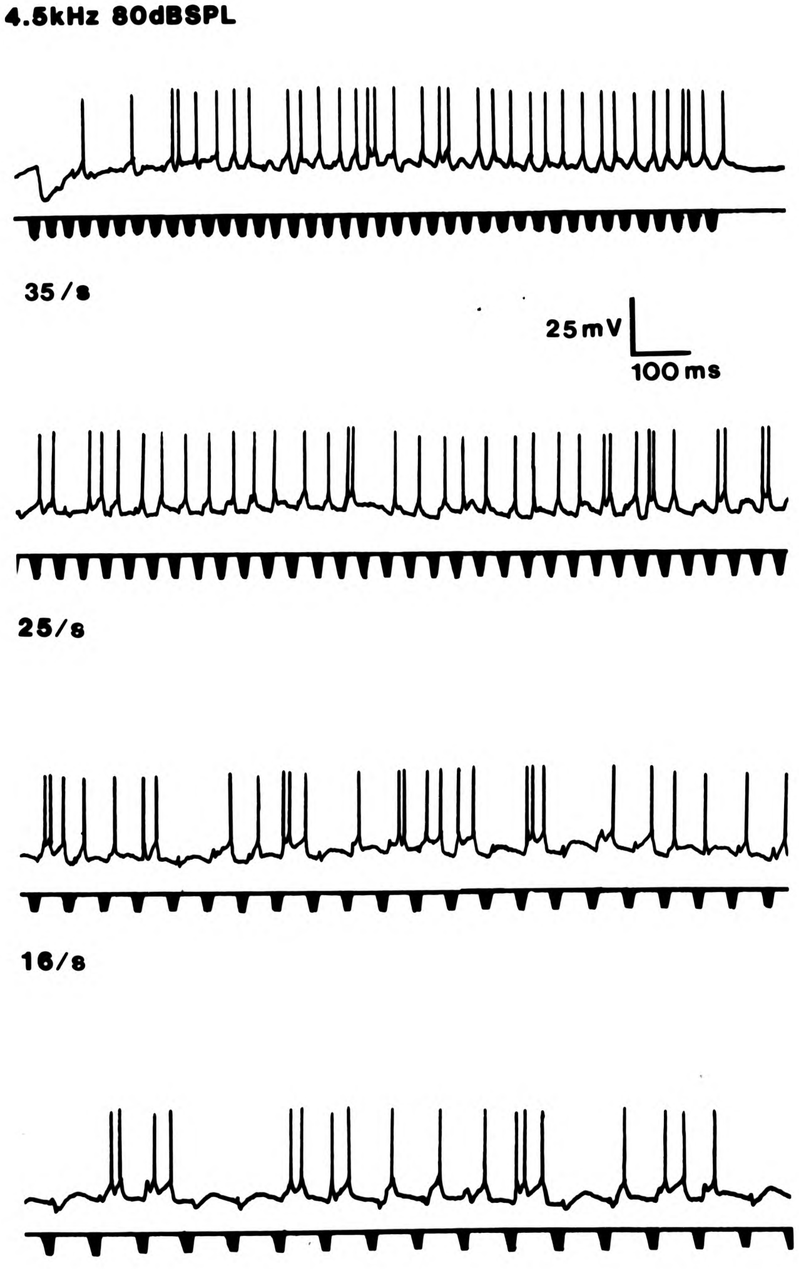


Fig. 3.16.1

The effect of intensity on the response of ANA to a simulation of the trill phrase of the courtship song, at the natural rate of 35 syllables/s.

The simulations contained 4.5kHz at intensities of,

84dB

80dB

78dB

68dB

58dB

58dB represents the threshold level for this neurone at this frequency.

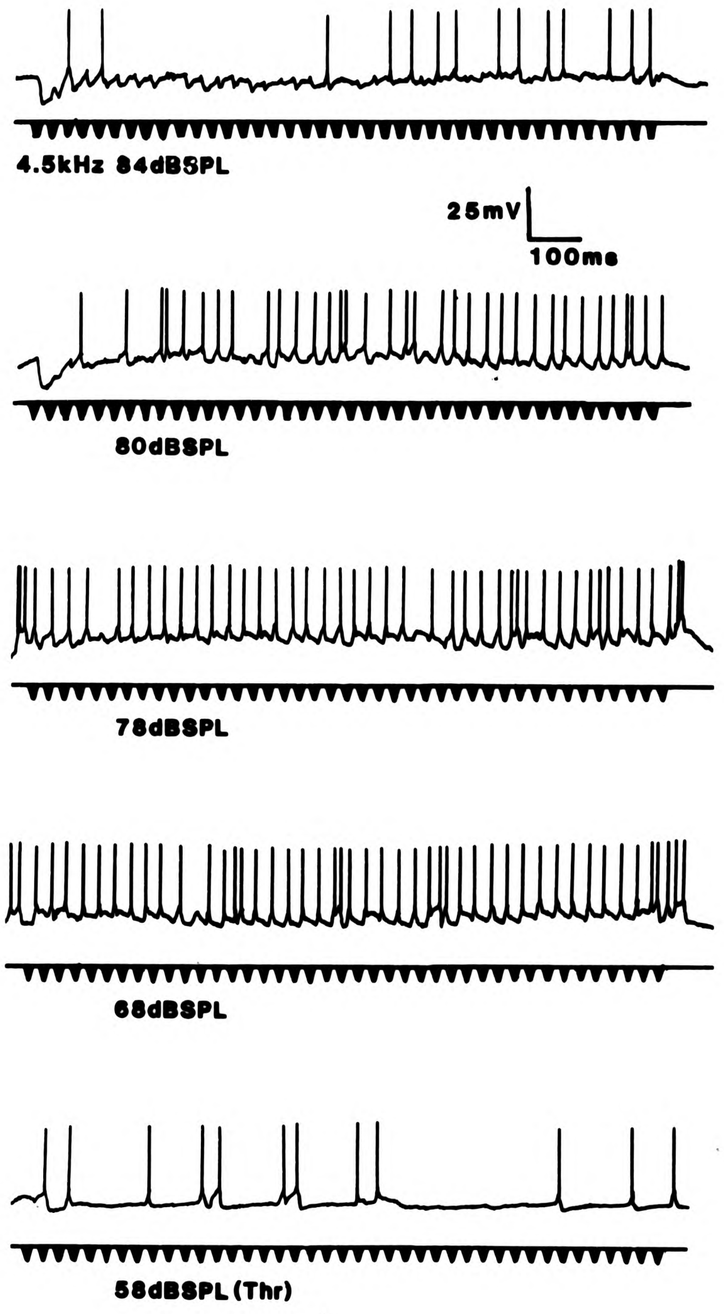


Fig. 3.16.2.

The effect of the frequency content of the stimulus on the response of ANA to a simulation of the trill phrase of the courtship song, at the natural syllable rate of 35/s.
The simulations contained,

4.5kHz at 83dB and 16kHz at 64dB.

Note: this represents the correct relative intensities of these frequencies in the natural song.

4.5kHz at 73dB and 16kHz at 64dB.

16kHz at 64dB.

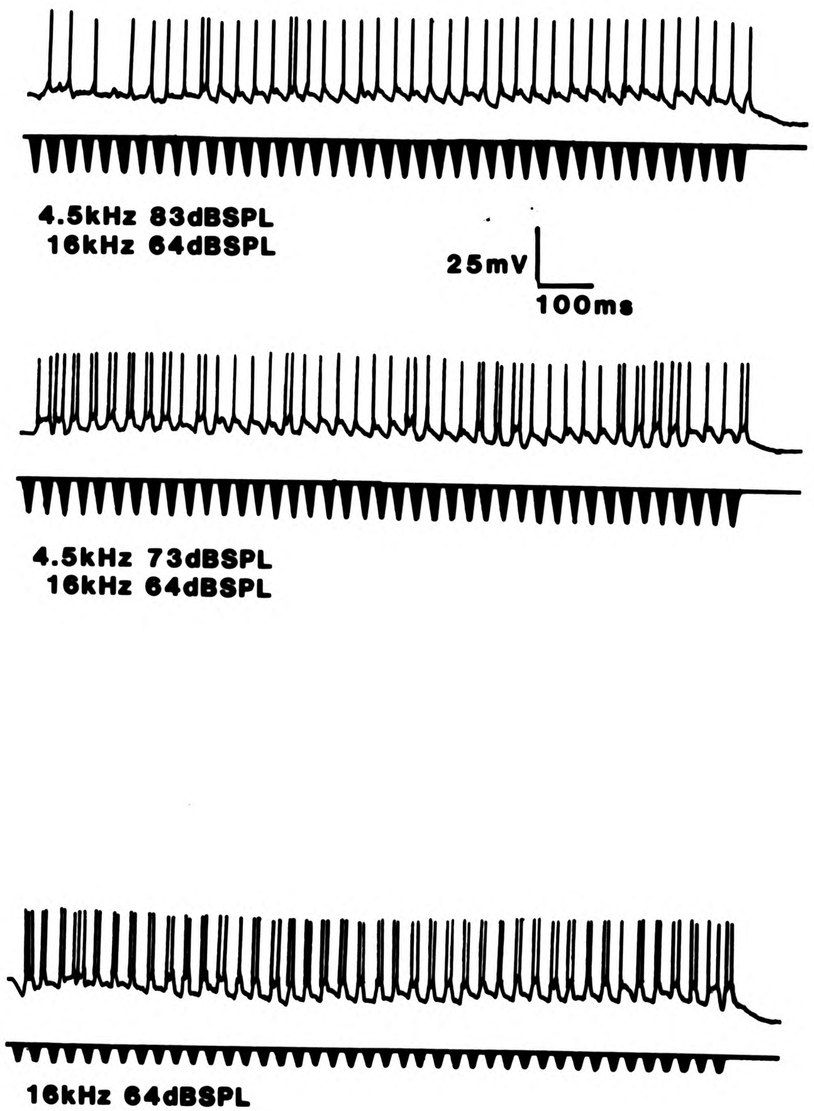
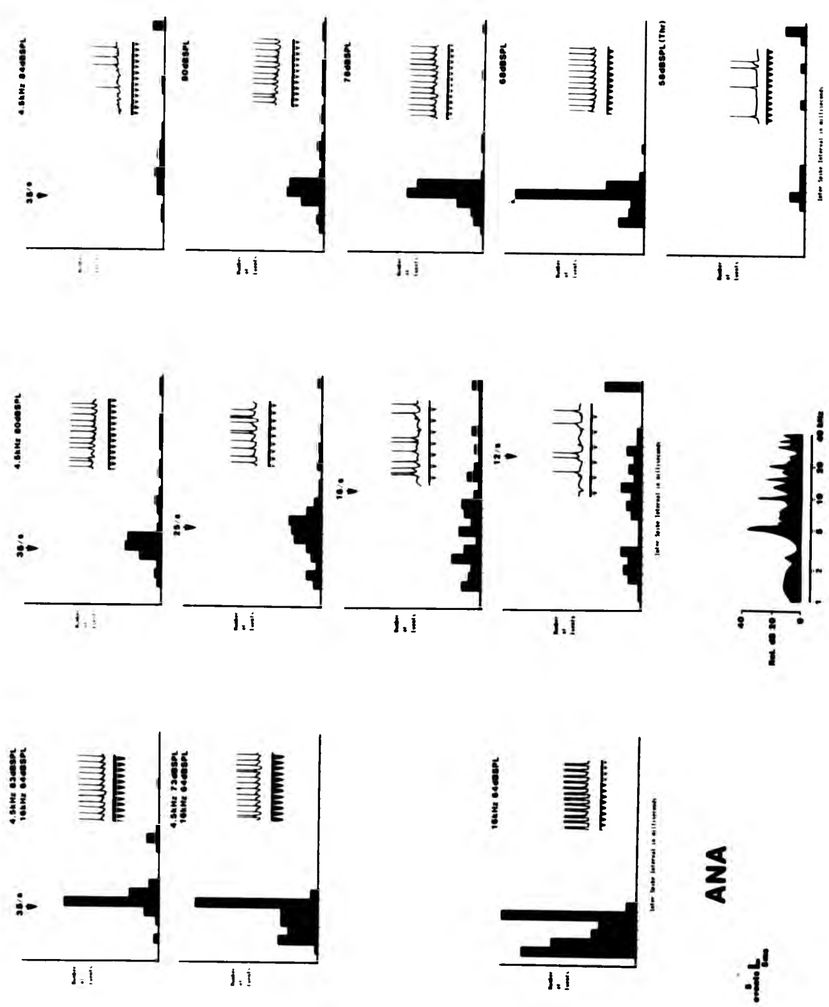


Fig. 3.16.3.

Interval histograms summarising the results shown in Fig. 3.16., 3.16.1. and 3.16.2. The interval histograms were derived from measurements (in ms) of the intervals between sucessive spikes of the responses of ANA to the frequency, rate and intensity changes shown as complete traces in the previous figures. The arrows indicate the rate of syllable presentation and are positioned with reference to the syllable interval. A correlation between the main peak of the histogram and the syllable rate indicates a response where each syllable tends to produce one spike.

The spectrogram at the bottom of the figure shows the relative intensities of the carrier frequency (4.5-5kHz) and the higher harmonics of the songs of T. oceanicus.



the natural song) produced a synchronised response in ANA. With a reduction in the intesity of the 4.5kHz component of the simulation the response of ANA was less synchronised in terms of one spike per syllable; many of the syllables were represented by a double spike response. 16kHz alone produced an average of 2/3 spikes per syllable. However this situation would not occur in the natural environment since none of the songs of T. oceanicus have a carrier frequency of 16kHz. The results from Fig. 3.16. are summarised in Fig. 3.16.3. This shows interval histograms, derived from measurements of the intervals (in ms) between successive spikes, from two presentations of each syllable rate, intensity change and frequency change.

3.2. Omega Neurones.

Intracellular recordings were carried out on two types of omega neurones: omega neurone 1 (ON1) and omega neurone 2 (ON2). Homologous neurones have been identified in Gryllus campestris and G. bimaculatus (Wohlers and Huber 1982) and ON1 has been previously described in Teleogryllus oceanicus (Casaday and Hoy 1977: Int-2). However this is the first identification of ON2 in this species.

3.2.1. Omega neurone 1.

Omega neurone 1 was recorded intracellularly in 41 preparations using glass micro-electrodes filled with Lucifer Yellow CH. After the physiological responses to

the test protocol had been recorded. Lucifer Yellow CH was injected into the cell using hyperpolarising current. This allowed anatomical identification of the neurone in each preparation.

3.2.1.1. Anatomy.

Omega neurone 1 is situated entirely within the prothoracic ganglion. Recordings from both sides of the ganglion show that these cells exist as a bilateral, mirror-image pair (Fig. 3.17). The preparation in Fig. 3.17.1 is a Lucifer yellow stain of a single ON1 recorded from the dendritic area ipsilateral to the cell body. Morphologically ON1 consists of two main dendritic areas situated in the auditory neuropile of each hemi-ganglion, connected via the omega shaped axon. None of the ON1 preparations showed a connection across the mid-line of the ganglion at the level of the two dendritic areas. The fibres in the dendritic area ipsilateral to the cell body have a smooth appearance indicating a post synaptic region whereas those in the contralateral area have a more beaded/blebbed appearance, characteristic of pre-synaptic dendrites (Romer and Marquart 1984). The cell bodies of the ON1 neurones lie in each anterior quadrant of the ganglion.

3.2.1.2. Physiology.

Threshold curves were calculated for Omega neurone 1 in 5 preparations. The frequency responses of the

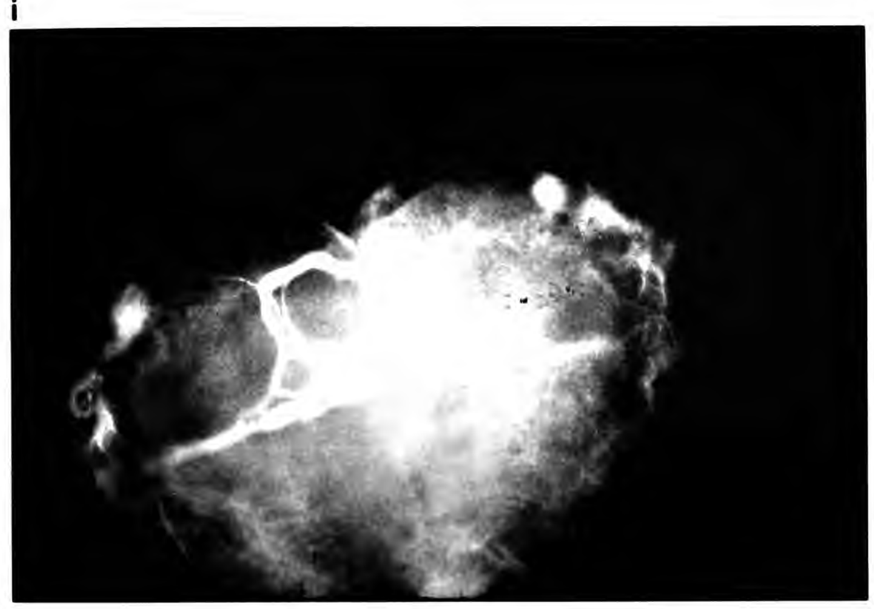
Fig. 3.17.

A camera lucida drawing of a double Lucifer Yellow CH stain of the mirror-image pair of Omega neurone 1s in the pro-thoracic ganglion.

The arrow indicates anterior.

Scale bar = 0.1mm

i



ii

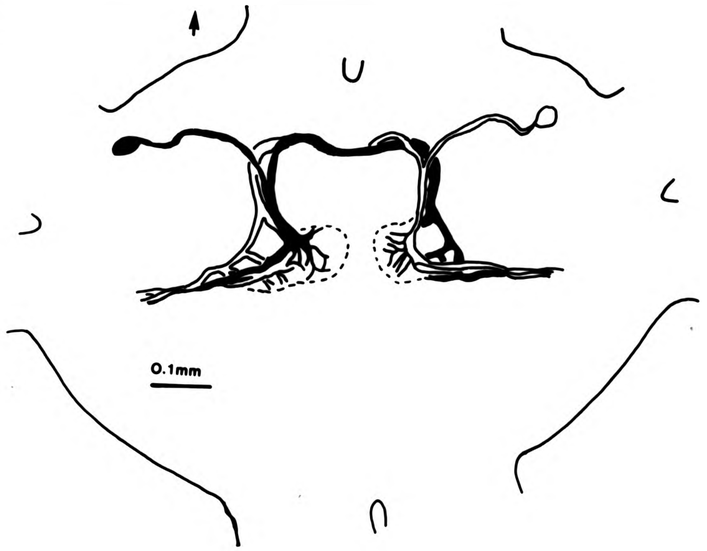


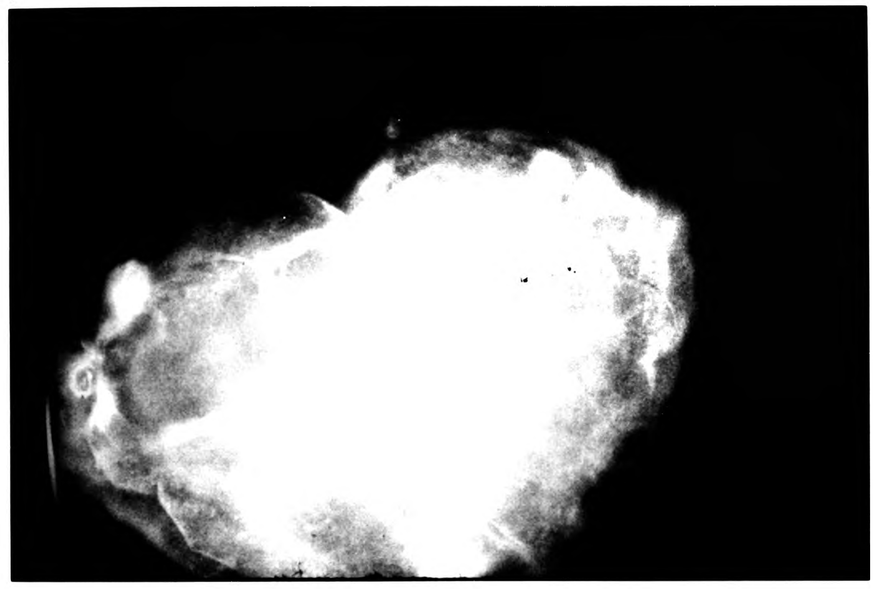
Fig. 3.17.

A camera lucida drawing of a double Lucifer Yellow CH stain of the mirror-image pair of Omega neurone 1s in the pro-thoracic ganglion.

The arrow indicates anterior.

Scale bar = Ø.1mm

i



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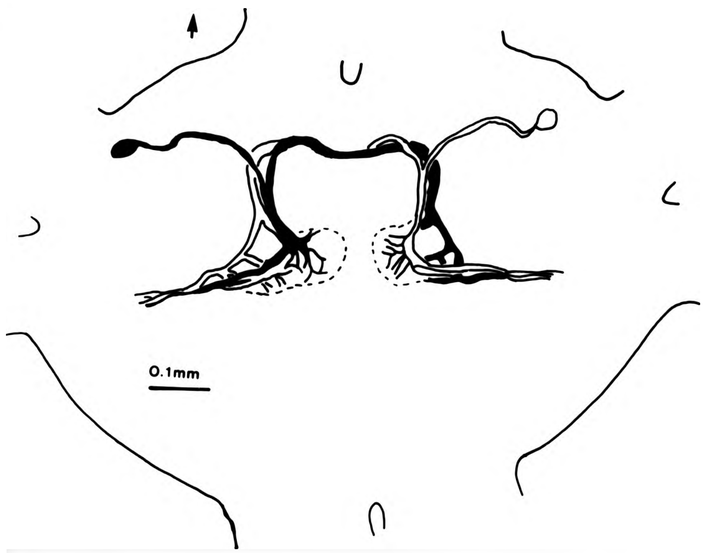

Fig. 3.17.1.

The morphology of Omega Neurone 1 (ON1) in the pro-thoracic ganglion. Ventral view.

(i) Photograph of a preparation stained with Lucifer Yellow CH. The dye was injected into the cell through an intracellular glass micro-electrode using hyperpolarising current.

(ii) A camera lucida drawing of this preparation. The arrow indicates the anterior direction. Scale bar = 0.1mm

Fig. 3.17.1.

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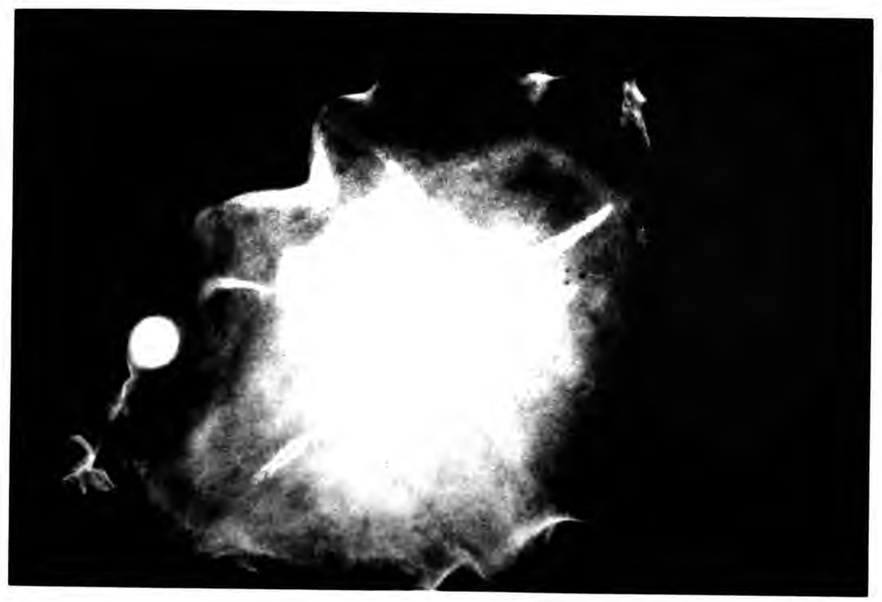


preparations were similar but there was some variation in the intensity sensitivities at some frequencies. An example of a threshold curve from one of the more sensitive units is shown in Fig. 3.18. In this preparation ON1 responded to frequencies between 2kHz and 100kHz and was particulary sensitive to 5kHz showing a threshold level at this frequency of 33dBSPL.

Intracellular recordings obtained from the dendritic area of ON1 ipsilateral to the cell body, in response to a low frequency tone (4.5kHz 84dB), consist of a train of action potentials on an underlying excitatory potential (EPSP). This is shown in Fig. 3.19(i) in which the unit produced an average of 9 spikes to each stimulus presentation. Spike rate is approximately 100/s. In this example the preparation was situated such that the sound stimulus was presented ipsilateral to the recording site. In an other preparation (Fig. 3.19(ii)) the sound stimulus was presented from the same side but the recording was taken from the dendritic area contralateral to the cell body. The response, recorded in this part of the neurone, consists only of action potentials; the EPSP does not travel the length of the axon. These results, together with the morphological differences observed between the two dendritic areas (Fig. 3.17.1), suggest that the dendritic area ipsilateral to the cell body is the input side of ONI and the contralateral area is the output side. However the recording in part (iii) of Fig. 3.19, obtained from another

Fig. 3.18.

Threshold curve of ON1.

Note: ON1 has a best frequency response at around 5kHz.

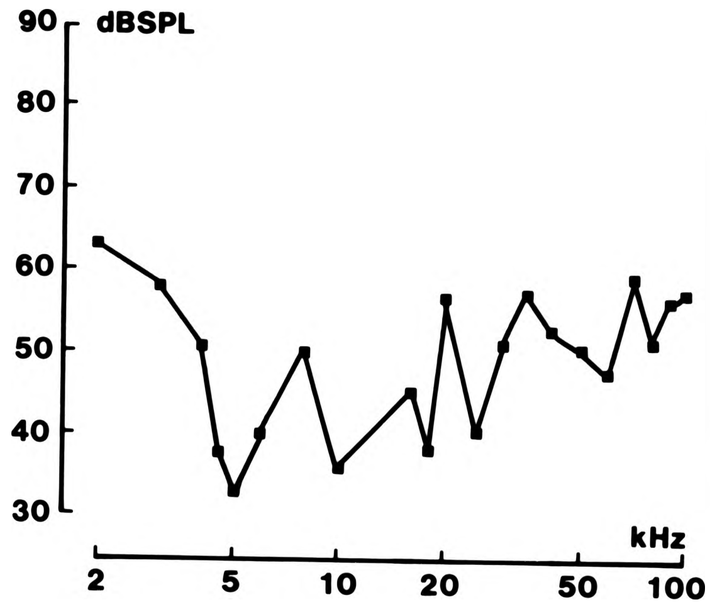


Fig 3.19.

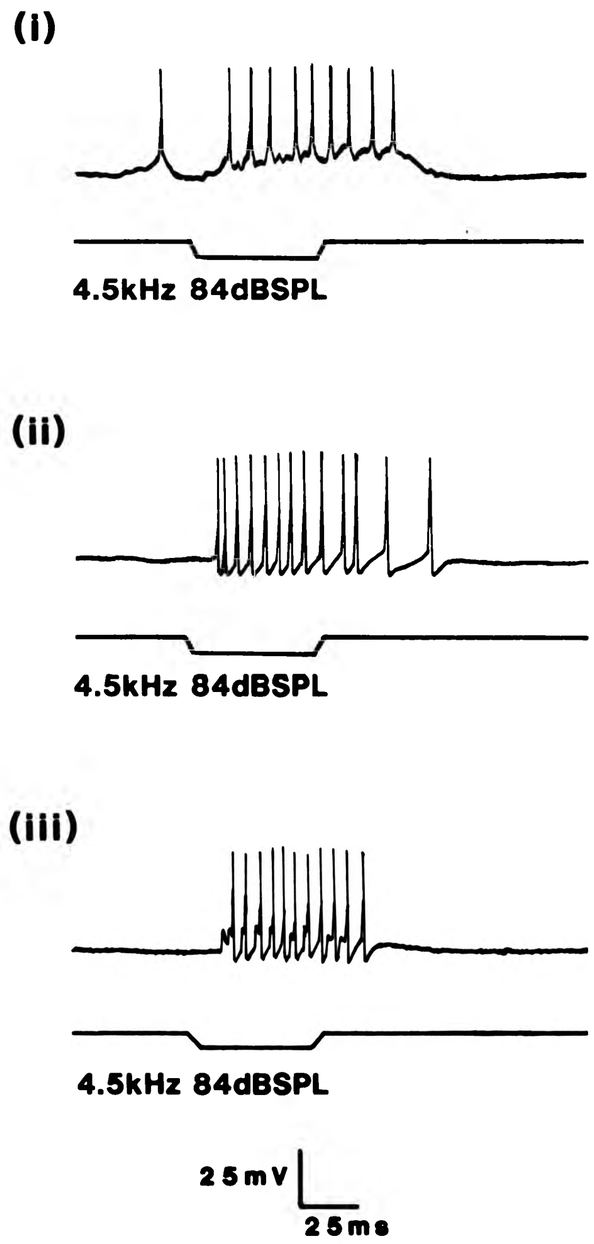
Intracellular recordings of the responses of ON1 to 4.5kHz at 84dBSPL with the electrode positioned in the main dendritic branch.

(i) ipsilateral to the cell body.

(ii) contralateral to the cell body.

(iii) contralateral to the cell body. Note: EPSP responses in addition to the spikes.

These recordings were taken from three different preparations.



preparation, was also taken from the dendritic area contralateral to the cell body in response to sound presented to this side of the preparation. Most of the response consists of action potentials but there is some EPSP activity underlying the spike responses and so the two dendritic areas may not be restricted to only input or output areas.

There is some anatomical evidence for the existence of input sites on the contralateral side. An intracellular recording of ON1 from the dendritic area ipsilateral to the cell body resulted in a complete fill of the neurone together with a primary neurone on the contralateral side (Fig. 3.20). Since the stained primary neurone was situated contralateral to the recording site, there was no possibility that the primary neurone was penetrated by the micro-electrode. In four other recordings of ON1 single primary neurones were stained in association with both the ipsilateral and contralateral dendritic fields (Fig. 3.20.1)

ON1 produces an excitatory response to frequencies between 2kHz and 100kHz and shows no evidence of inhibitory post synaptic potentials (IPSP) in response to any of these frequencies. However when tested throughout the intensity range at 4.5kHz ON1 showed a decrease in response high intensities as shown in Fig. 3.21(i). in which the recording was taken from the dendritic area contralateral to the cell body. The threshold response of this unit to

Fig. 3.20.

The morphology of ON1 and a primary neurone associated with its contralateral dendritic field.

(i) Photograph of ON1, stained by the injection of Lucifer Yellow CH through a glass micro-pipette situated in the dendritic area ipsilateral to the cell body. As a result of staining the ON1 neurone, a primary neurone associated with the contralateral dendritic area was also stained.

(ii) A camera lucida drawing of this preparation.

PA = Primary Axon.

PD = Primary Dendrites.

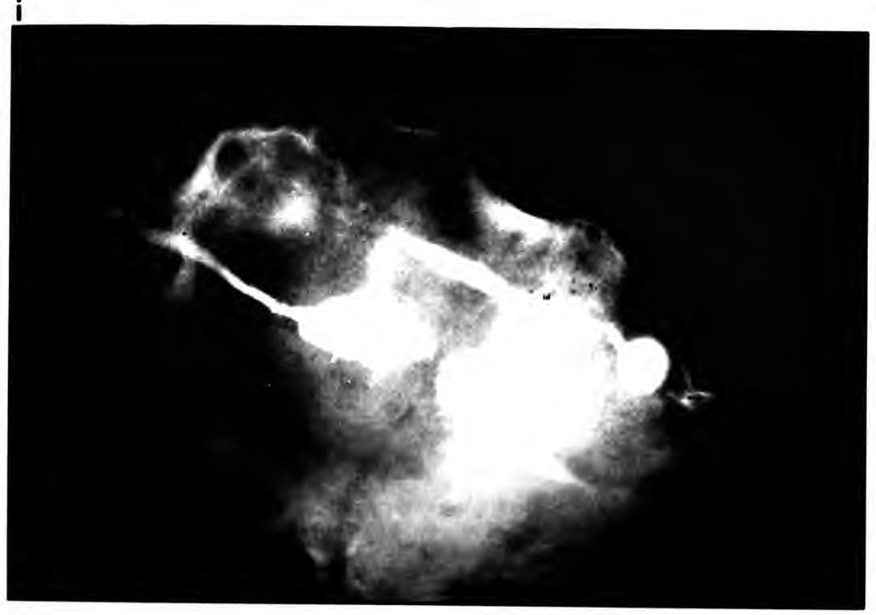


Fig. 3.20.

The morphology of ON1 and a primary neurone associated with its contralateral dendritic field.

(i) Photograph of ON1, stained by the injection of Lucifer Yellow CH through a glass micro-pipette situated in the dendritic area ipsilateral to the cell body. As a result of staining the ON1 neurone, a primary neurone associated with the contralateral dendritic area was also stained.

(ii) A camera lucida drawing of this preparation.

PA = Primary Axon.

PD = Primary Dendrites.

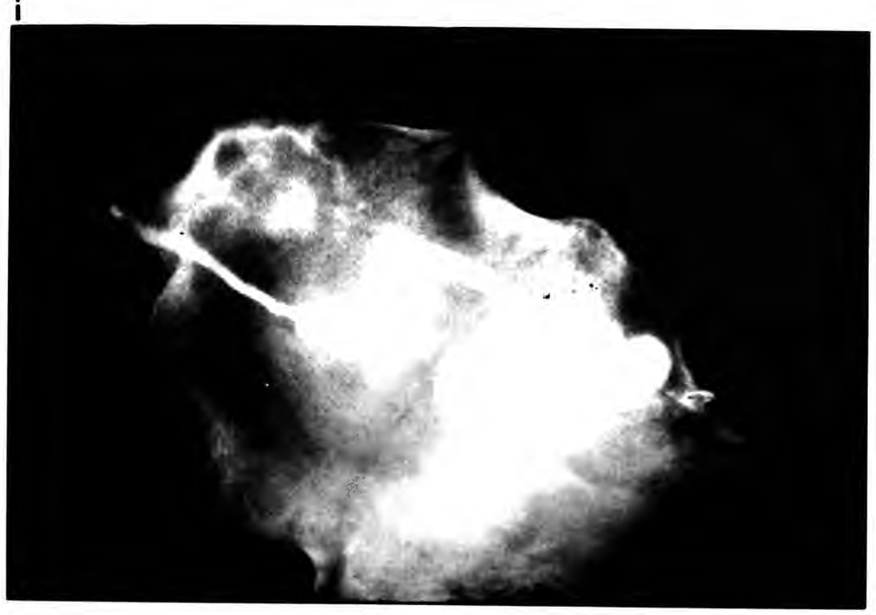


Fig 3.20.1.

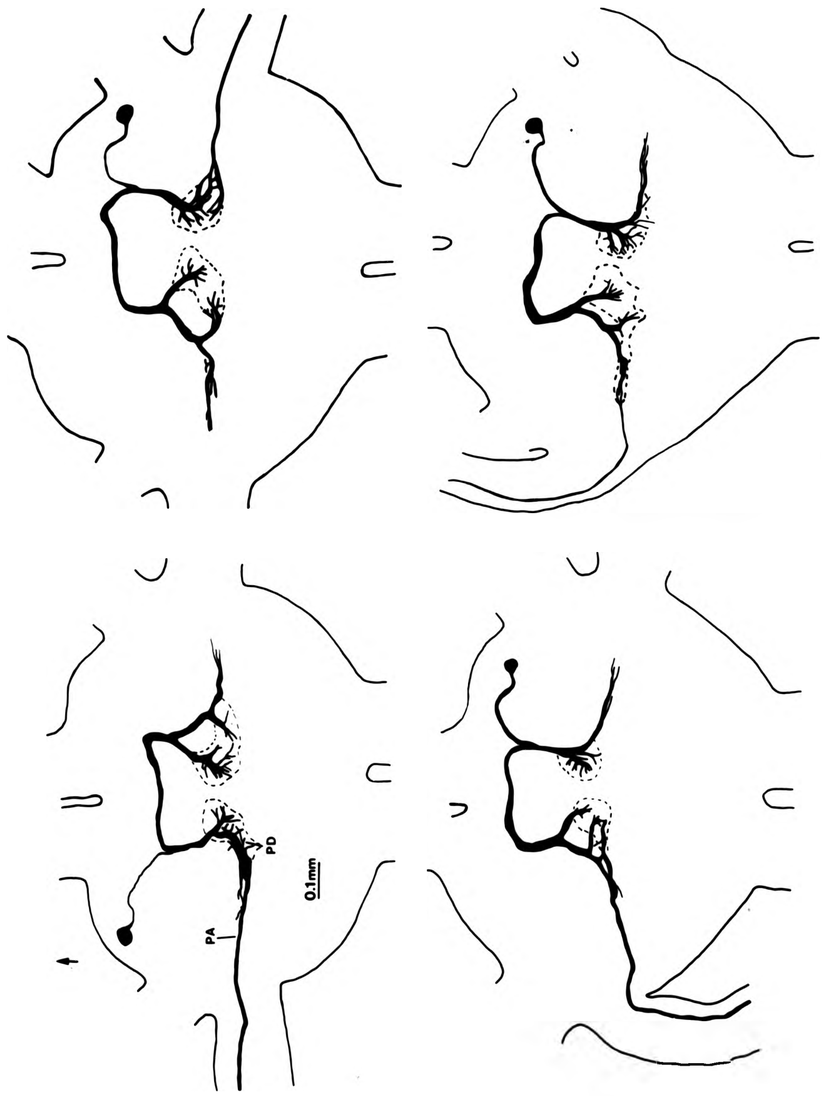
Camera lucida drawings of Lucifer Yellow CH stains of four ON1 neurones together with primary neurones associated with,

the ipsilateral dendritic area of ON1 (upper).

the contralateral dendritic area of ON1 (lower drawings).

PA = Primary Axon.

PD = Primary Dendrites.



4.5kHz was about 40dBSPL. An increase of 4dBSPL above this level (44dB)produced a response which consisted of an average of 2.5 spikes to each presentation of the stimulus. With an increase in intensity to 54dBSPL the neurone's response increased to an average of 7 spikes per stimulus. The response increased to 11.5 spikes with a further increase in intensity to 64dBSPL but at 10dB above this level the reponse showed a reduction in spike number: 74dBSPL produced an average of 8 spikes to each stimulus. With a further increase in intensity to 84dBSPL, which was the highest intensity tested, the response was reduced to an average of 3.5 spikes per stimulus. To determine whether the decrease in response was the result of fatigue in the neuron or due to the presence of a high intensity inhibitory input this neurone was tested with the same protocol, ten minutes after application of 5×10^{-7} M Picrotoxin. The results are shown in Fig. 3.21(iii). Before the application of Picrotoxin the unit's response to 4.5kHz from 44dBSPL to 84dBSPL formed a bell shaped curve. Ten minutes after the application of Picrotoxin the neurone's response to 4.5kHz at 84dBSPL increased by an average of 5 spikes. These results suggest that the reduction in the response of ON1 when presented with high intensities of 4.5kHz is the result of an inhibitory input which can be reduced by the application of picrotoxin. If so then presumably a longer application, or a higher concentration of the drug would have shown a complete block of the

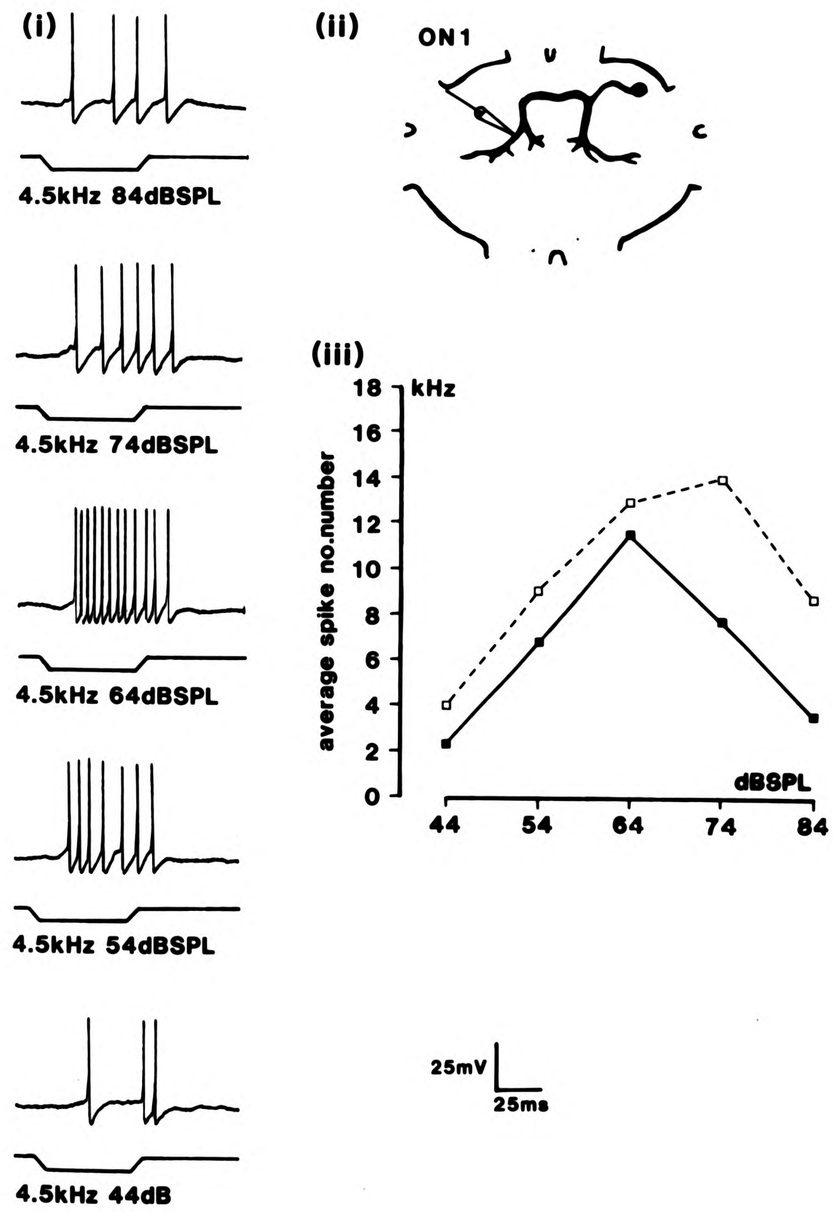
Fig. 3.21.

(i) Intracellular recordings of the responses of ON1 to a 4.5kHz tone presented at 44dB, 54dB, 64dB, 74dB and 84dB.

(ii) Diagramatic representation of ON1 showing the location of the recording electrode.

(iii) The effect of Picrotoxin (5×10^{-7} M) on the response of ON1 to 4.5kHz presented at 44dB, 54dB, 64dB, 74dB and 84dB. Each point on the graph represents the average spike number produced by ON1 in response to 4.5kHz at the intensity shown. The solid line represents the response before Picrotoxin. The broken line represents the response, ten minutes after the application of Picrotoxin.

Note: The effect of picrotoxin was to increase the spike number, particularly, in response to the high intensities.



inhibitory response.

To investigate the possible role of ON1 in song recognition the neurone was presented with simulations of the three different song types. The responses of ON1 to simulations containing 4.5kHz at 89dB plus 16kHz at 70dB are shown in Fig. 3.22. The intracellular recording electrode was located in the dendritic area contralateral to the cell body (Fig. 3.22(i). ON1 produced an accurately synchronised response to the syllable patterns of the courtship song, calling song and aggression song consisting of 2/3 spikes per syllable.

3.2.2. Omega neurone 2.

Intracellular recordings were made from Omega neurone 2 in six preparations, using glass micro-electrodes filled with Lucifer Yellow CH. The neurone was anatomically identified as ON2 in all the preparations and physiological information was recorded in five out of the six. In one preparation, Omega neurone 1 with its cell body in the same anterior quadrant as the cell body of ON2, was also stained which shows that these neurone co-exist in the ganglion and ON2 is not an aberrant form of ON1.

3.2.2.1. Anatomy.

Omega neurone 2 is situated entirely within the prothoracic ganglion. Recordings from both sides of the ganglion show that ON2 exists as a bilateral mirror-image

Fig. 3.22.

Intracellular recordings of the responses of ON1 to simulations of the three different song types.

(i) Diagramatic representation of ON1 in the Pro-thoracic ganglion to show the location of the recording electrode.

The responses of ON1 to simulations of the

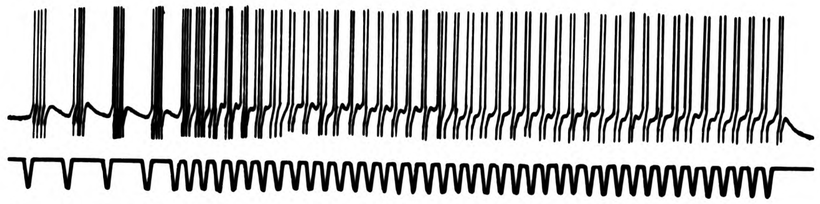
(ii) courtship song,

(iii) calling song,

(iv) aggression song,

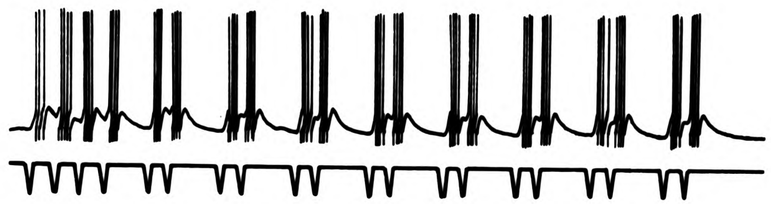
with each simulation containing 16kHz at 70dB plus 4.5kHz at 89dB.

(ii) courtship song

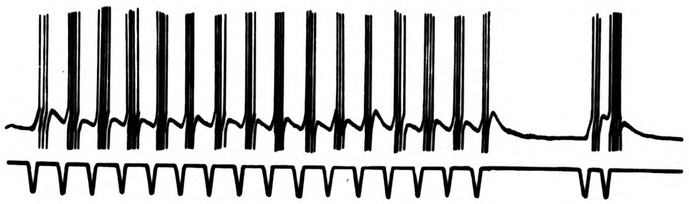


16kHz70dBSPL 4.5kHz89dBSPL

(iii) calling song



(iv) aggression song



pair. Fig. 3.23 shows a Lucifer Yellow stain of ON2. As with ON1, ON2 is named because of its omega shape consisting of two dendritic areas situated in the auditory neuropil of each hemi-ganglion connected via the axon. The main anatomical difference between the two omega neuron types is that ON2 has a large process, from the dendritic area ipsilateral to the cell body, which projects across the mid-line to the contralateral dendritic area. When viewed in whole mount this projection appears to lie ventral to the contralateral dendritic field. The diameter of the axon in ON2 is approximatly 1/4 the diameter of the axon in ON1. ON2 shows no clear distinction between the two dendritic areas in terms of a smooth or beaded appearance to the fibres. The cell bodies of ON2 are situated in each anterior quadrant of the ganglion, adjacent to the cell bodies of ON1. In one preparation, a primary neurone was filled in association with the dendritic area ipsilateral to the cell body (Fig. 3.24).

3.2.2.2. Physiology.

Physiological information on ON2 was recorded in five preparations. In each recording the electrode was positioned within the large dendritic projection, towards the centre of the ganglion. On penetration, all five examples of ON2 showed a membrane potential of approximately -40mV; the membrane potential for both ON1 and ANA is approximately -60mV. ON2 was tested in response

Fig. 3.23.

The morphology of Omega Neurone 2 (ON2) in the Pro-thoracic ganglion. Ventral view.

(i) Photograph of a preparation stained with Lucifer Yellow CH. The dye was injected into the cell through an intracellular glass micro-electrode using hyperpolarising current.

(ii) A camera lucida drawing of this preparation. The arrow indicates the anterior direction. Scale bar = 0.1mm.

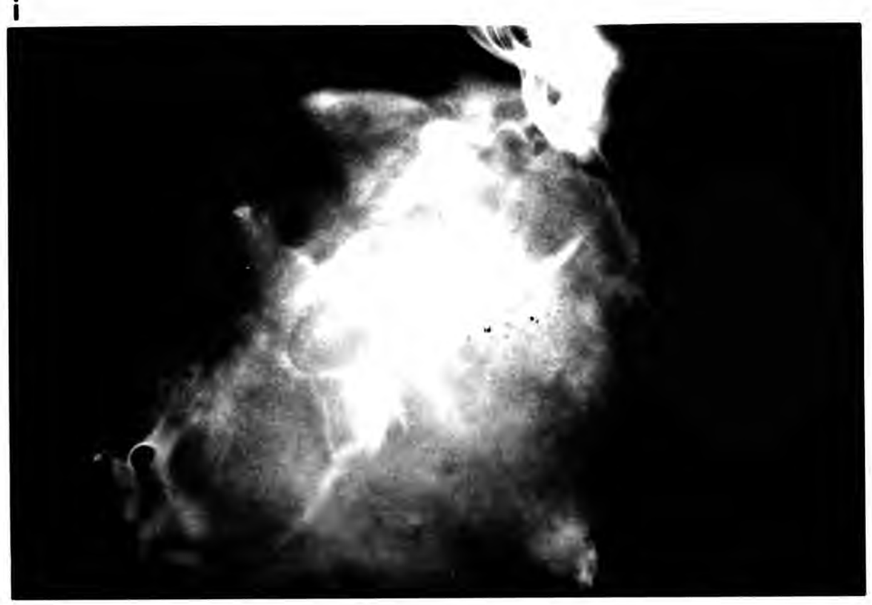


Fig. 3.23.

The morphology of Omega Neurone 2 (ON2) in the Pro-thoracic ganglion. Ventral view.

(i) Photograph of a preparation stained with Lucifer Yellow CH. The dye was injected into the cell through an intracellular glass micro-electrode using hyperpolarising current.

(ii) A camera lucida drawing of this preparation. The arrow indicates the anterior direction. Scale bar = 0.1mm.

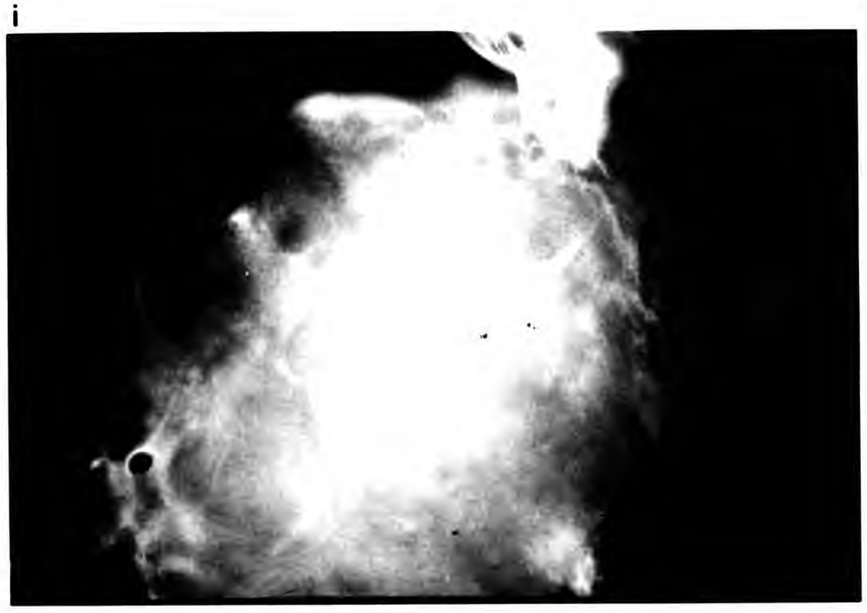


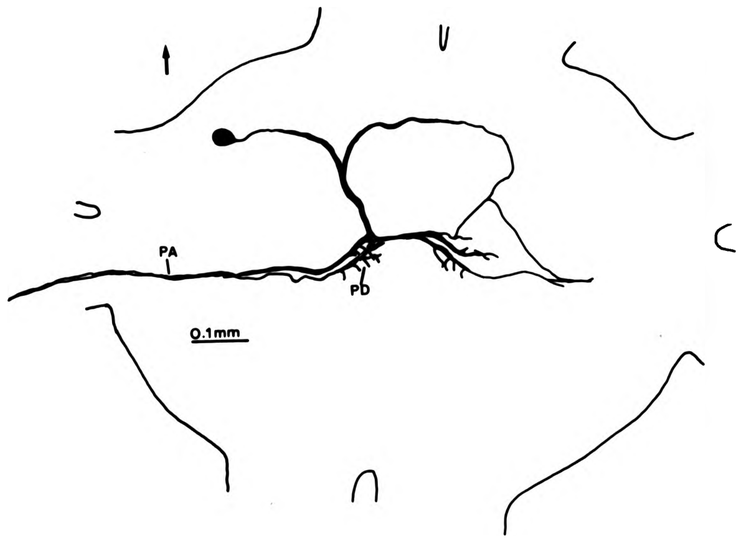
Fig. 3.24.

A camera lucida drawing of the morphology of ON2 and a primary neurone associated with the dendritic area of ON2 ipsilateral to the cell body.

PA = Primary Axon.

PD = Primary Dendrites.

Arrow indicates the anterior direction.



to a range of intensities of frequencies between 2kHz and 80kHz. Each response consisted of a compound excitatory post synaptic potential but none of the frequencies produced action potentials. Fig. 3.25 shows the typical responses of ON2 to 4.5kHz, 16kHz and 30kHz over a range of intensities. The amplitude of the excitatory potential produced in response to each of these frequencies increased as the intensity of the tone increased. In response to 16kHz at 80dB the amplitude of the response was around 25mV. At comparable intensities the amplitude of the EPSP was greater in response to 16kHz and 30kHz than to 4.5kHz which suggests that this neurone, unlike ON1, would have a best frequency above 4.5kHz.

Two of the five preparations, showed a smaller amplitude of response to 4.5kHz at 84dBSPL than to this frequency at 74dBSPL. These preparations also showed a decrease in the response to 16kHz when it was presented simultaneously with a high intensity 4.5kHz tone. None of the five preparations showed an identifiable inhibitory post-synaptic potential in response to any of the frequencies tested.

An attempt was made to induce spiking in ON2 by injecting the neurone with depolarising current in the presence of 16kHz at 80dBSPL. However even 10nA of depolarising current failed to produce spikes in the neurone. Therefore, functionally, ON2 is likely to be a non-spiking neurone.

Fig. 3.25.

Physiology of ON2.

Intracellular recordings of the response of ON2 to,

(i) 4.5kHz at intensities of 64dB, 74dB and 84dB.

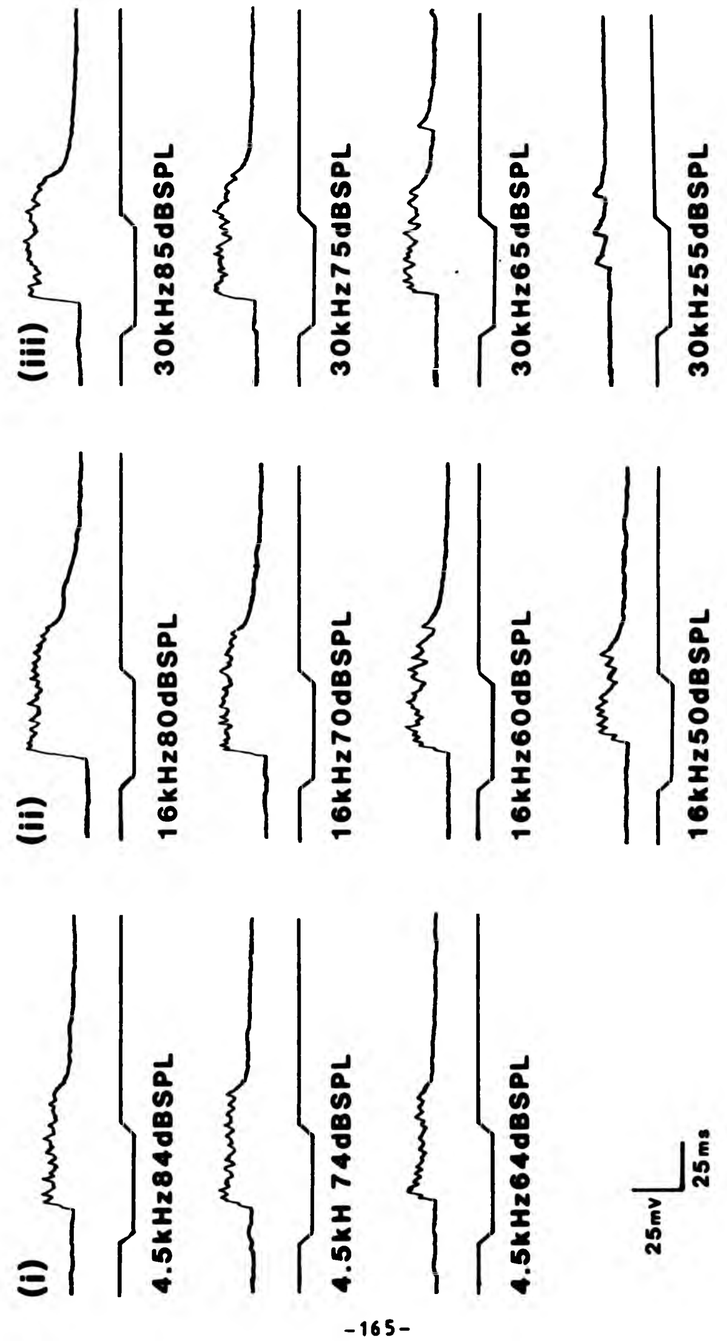
(ii) 16kHz at intensities of 50dB, 60dB, 70dB and 80dB.

(iii) 30kHz at intensities of 55dB, 65dB, 75dB and 85dB.

Upper trace represents the neural response.

The deflection of the lower trace represents the stimulus.

Note: no spiking activity was produced in response to any intensity of the frequencies tested.



Three preparations were presented with simulations of the courtship, calling and aggression songs containing 4.5kHz at 84dBSPL (Fig. 3.26). In two of these the excitatory potential shown by ON2 was correlated with the temporal patterns of all three songs; in the other preparation the calling and aggression songs produced a synchronised response but the courtship trill phrase did not.

In addition to the ascending neurones and the omega neurones there are two other groups of auditory interneurone which exist in the pro-thoracic ganglion: through neurones (TN) and descending neurones (DN). Examples of both of these were recorded and stained during the investigations into the structure and function of the ascending and omega neurones.

3.3. Through neurones (TN).

In one preparation an example of a through neurone was recorded, extracellularly, and stained using cobalt chloride filled electrodes. Through neurones were often partially filled while extracellular recordings were being carried out on the ascending neurones but in this example the recordings were taken from the through neurone. This recording lasted about twenty minutes which was sufficient to allow identification of the cell but was not long enough to produce a complete fill.

Fig. 3.26.

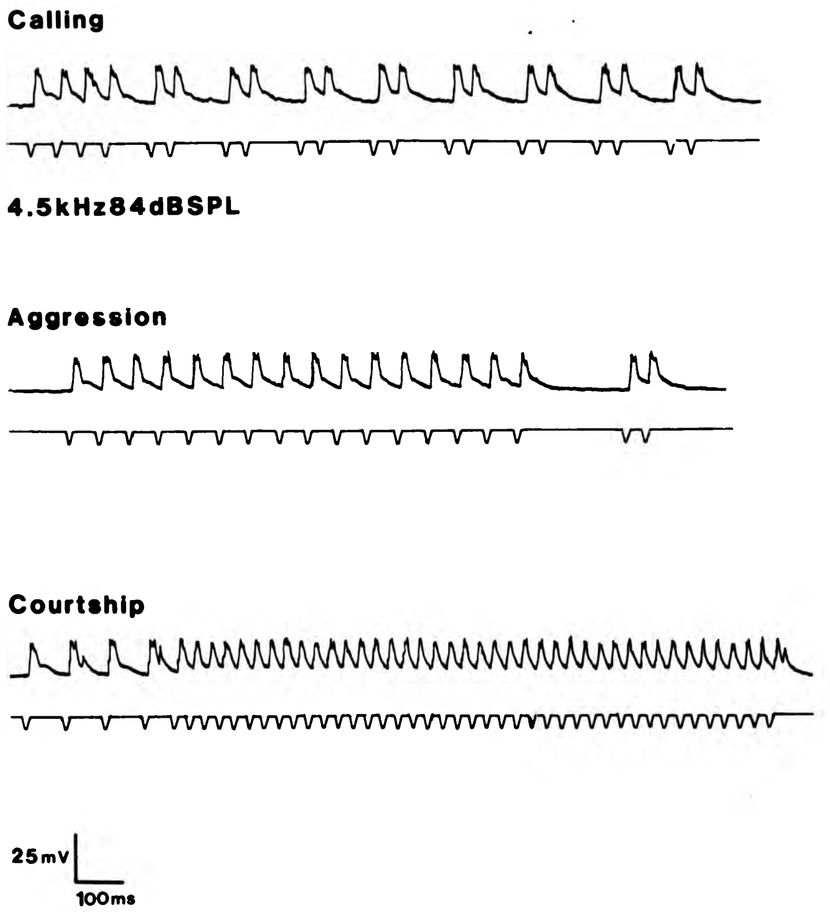
Intracellular recordings of the response of ON2 to simulations of the three song types, each containing 4.5kHz at 84dB,

(i) calling song,

(ii) aggression song,

(iv) courtship song.

Note: the EPSP activity is correlated with the syllable patterns of each song.



3.3.1. Anatomy.

The morphology of this through neurone within the prothoracic ganglion is shown in Fig. 3.27. The axon passes through this ganglion from the connectives between the meso-thoracic and pro-thoracic ganglia, and then ascends towards the supra-oesophageal ganglion. Within the pro-thoracic ganglion, dendritic arborizations project laterally and dorsally from both sides of the axon. Some cross the mid-line while others are restricted to the ipsilateral side. One arborization projects out towards the leg nerve ipsilateral to the axon. There is no stain of the neurite or cell body of this neurone which indicates that it may lie in another ganglion. However, the cell body may not have been stained because the recording time was insufficient to result in a complete stain of this neurone.

3.3.2. Physiology.

The threshold curve for this through neurone (Fig. 3.27(ii)) showed a response from 8kHz up to 100kHz. There was insufficient data to plot a threshold of inhibition curve for this neurone but the dot raster displays in part (iii) of Fig. 3.27 show two-tone suppresion occurs. When presented with 40kHz at 76dB, an average of 10.6 spikes were produced in response to eight presentations of the stimulus but with the simultaneous presentation of a high intensity 3kHz (90dB) tone this was reduced to an average of 2 spikes to each presentation of the stimulus.

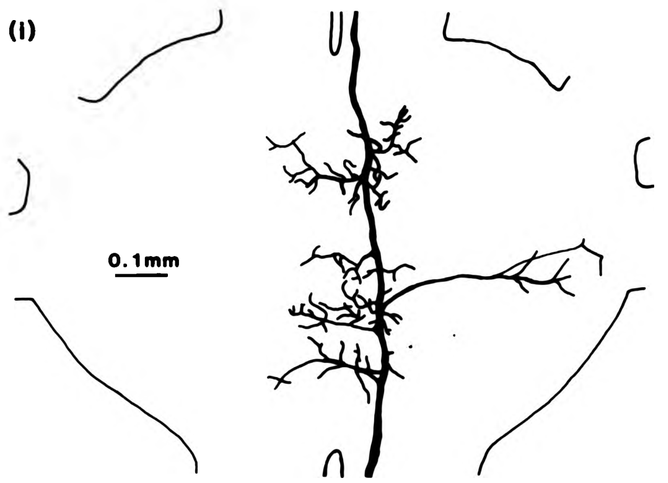
Fig. 3.27.

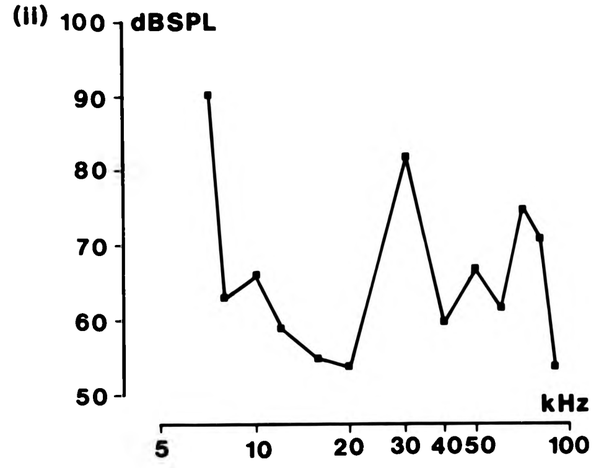
Morphology and physiology of a through neurone (TN) within the Pro-thoracic ganglion.

(i) A camera lucida drawing of a TN from a cobalt chloride stain.

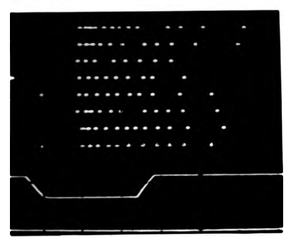
(ii) Threshold curve for this preparation.

(iii) The effect of two tone stimulation on TN.
Dot raster displays showing the response of a TN to 40kHz at 76dB presented alone and together with 3kHz at 90dB. Each dot represents one spike and each trace shows the responses to eight presentations of the stimulus. The stimulus is represented by the deflection of the lower trace.

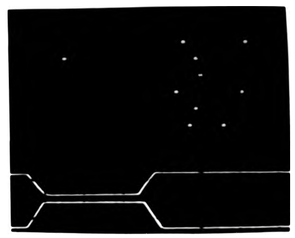




(iii)



40kHz76dBSPL



40kHz76dBSPL
+3kHz90dBSPL

3.4. Descending Neurones (DN).

Two examples of descending neurones were identified anatomically using Lucifer Yellow filled electrodes but physiological information was obtained from only one preparation.

3.4.1. Anatomy.

One of the early intracellular experiments resulted in a stain of a descending neurone (Fig. 3.28). Morphologically this neurone consists of two dendritic areas in the posterior half of the prothoracic ganglion. The first, smaller branch, lies ipsilateral to the descending axon and projects towards the the anterior of the ganglion. The second dendritic branch crosses the mid-line and arborises extensively within the contralateral hemi-ganglion with projections towards the leg nerve contralateral to the axon; towards the anterior of the ganglion and back across the mid-line. The cell body lies in the posterior quadrant of the ganglion contralateral to the descending axon.

Morphologically, the second type of descending neurone (Fig. 3.29(i)) consists of a main dendritic area which projects towards the leg nerve ipsilateral to the descending axon. In addition, a circular dendritic projection crosses the mid-line and connects to the axon below its main branching point. The cell body lies in the anterior quadrant of the ganglion, contralateral to both

Fig. 3.28.

The morphology of a descending neurone in the prothoracic ganglion. A camera lucida drawing of a Lucifer Yellow CH stain. Ventral view.

Note: the cell body is located in the posterior quadrant contralateral to the descending axon.

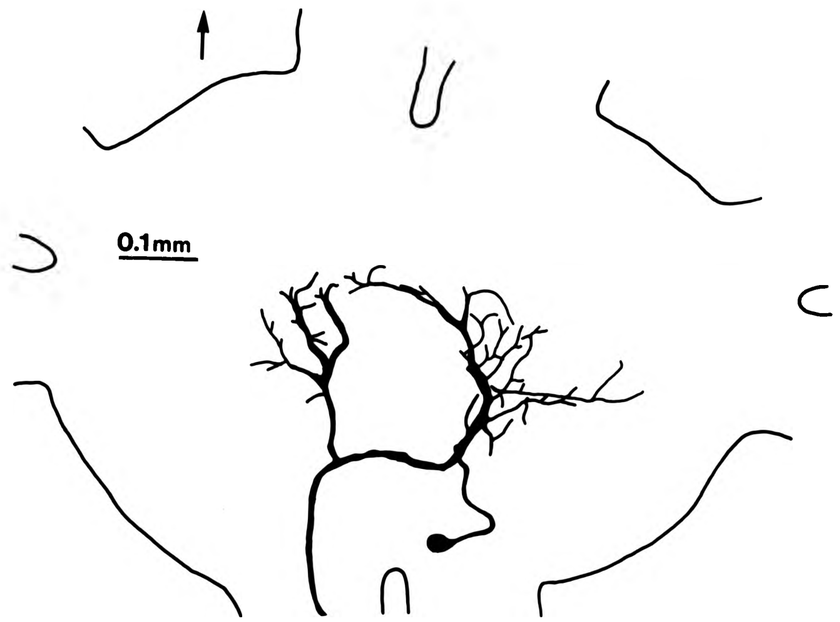


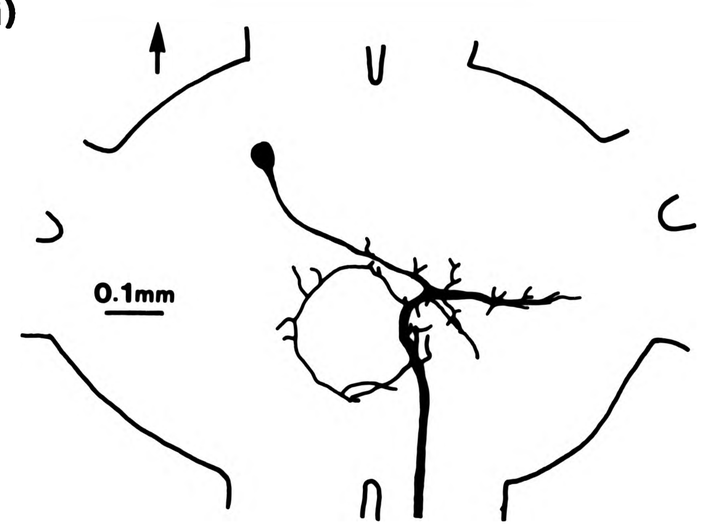
Fig. 3.29.

(i) The morphology of a descending neurone (DN1) in the Pro-thoracic ganglion.

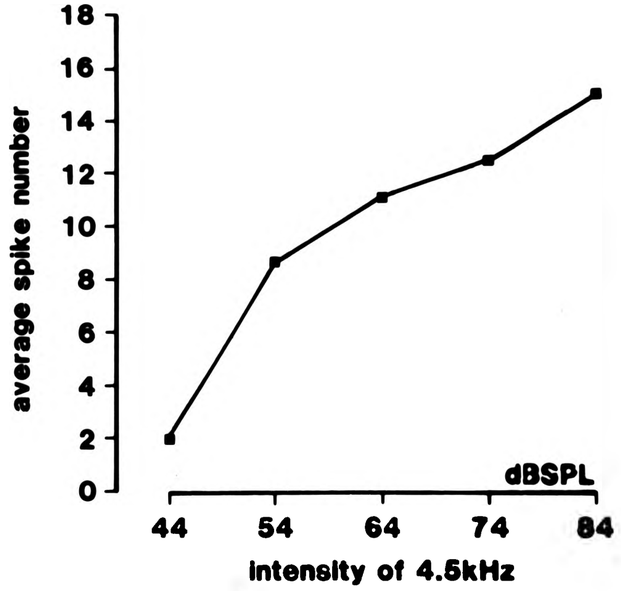
A camera lucida drawing of a Lucifer Yellow stain of this neurone. Arrow indicates the anterior direction.

(ii) The responseS of DN1 to a 4.5kHz tone presented at 44dB, 54dB, 64dB, 74dB and 84dB. Each point on the graph represents the average spike number produced in response to the stated stimulus over four presentations.

(i)



(ii)



the descending axon and the dendritic branch.

3.4.2. Physiology.

The descending neurone in Fig. 3.28 was identified as an auditory neurone by its correlated spiking response to auditory stimuli but unfortunatly no physiological data was recorded from this neurone. However the responses of the neurone in Fig. 3.29 to a 4.5kHz tone at a range of intensities were recorded. These are shown in Fig. 3.29(ii). This neurone produced a burst of excitation consisting of an average of 15 spikes when presented with 4.5kHz at 84dBSPL ipsilateral to its main dendritic area. Tested over a range of intensities this descending neurone shows no high intensity inhibition: as the intensity of the tone increases the number of spikes in the response also increases. The threshold level at this frequency is just below 44dBSPL.

3.5. Double Electrode Recording Experiments.

A series of experiments were carried out to investigate the origin of the neurally mediated inhibitory responses shown by ANA to low frequency tones. The existance of synaptic connections between two neurones can be investigated, physiologically by monitoring the responses of one neurone while the response of the other neurone is manipulated by the injection of current through an intracellular electrode. If the activity of the

monitored neurone is altered as a result of manipulation of the response of the impaled neurone and the latency between the two responses is short then there is likely to be synaptic connections between these two cells.

In the double electrode experiments a suction electrode on the cervical connective was used to monitor the response of ANA. To establish that the large spikes recorded in the neck connective were attributable to ANA experiments were carried out in which the activity of ANA was recorded in a suction electrode and a glass micro-electrode, simultaneously. The glass microelectrode was used to impale ANA in the main dendritic area close to its branching point with the axon and the suction electrode was placed over the cut end of the ipsilateral cervical connective. Fig. 3.30(i) shows the positions of the two electrodes. In part (ii) the responses recorded from both electrodes to a 12kHz tone at 69dBSPL are compared. This stimulus produced 10 spikes in each trace and the responses were correlated in terms of spike number and response duration. Therefore, responses from the same neurone were being recorded in both electrodes.

This neurone was identified by injecting the impaled cell with the fluorescent dye Lucifer Yellow using hyperpolarising current. After processing the preparation was viewed under ultra violet light and the anatomy of ANA revealed confirming that this was the neurone being recorded by both the intracellular and suction electrodes.

Fig. 3.30.

Double electrode recordings from ANA.

(i) A camera lucida drawing of a Lucifer Yellow CH stain of ANA showing the location of the two recording electrodes.

se = suction electrode, which records the extracellular responses of ANA from its axon in the cervical connective.

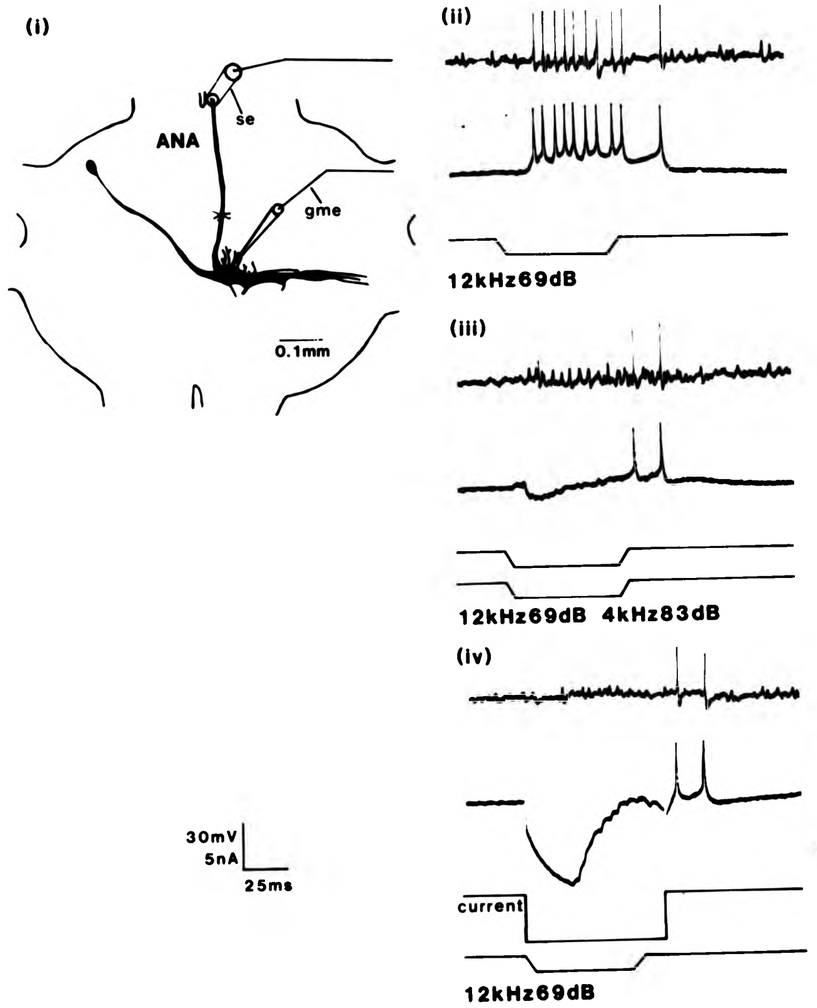
gme = glass micro-electode, which records the intracellular responses of ANA from its main dendritic branch in the prothoracic ganglion.

The extracellular (upper trace) and intracellular (middle trace) recording of the response of ANA to,

(ii) 12kHz at 69dB.

(iii) 12kHz at 69dB and 4kHz at 83dB.

(iv) 12kHz at 69dB and injection of 5nA of hyperpolarising current.



This experiment was carried out in three preparations and each confirmed the presence of ANA in the suction electrode. At the beginning of each double electrode experiment the neurone recorded in the suction electrode was tested with low frequency tones and physiologically identified as ANA. The experiments in parts (iii) and (iv) of Fig 3.30 were carried out to show that it is possible to manipulate the responses of an intracellularly recorded neurone by current injection. Fig. 3.30(iii) shows that the response of ANA to 12kHz is inhibited by the simultaneous presentation of 4kHz at 83dBSPL. The same effect can be achieved by suppressing the 12kHz response with 5nA of hyperpolarising current (Fig. 3.30(iv)). The changes in the response of ANA were monitored by the suction electrode.

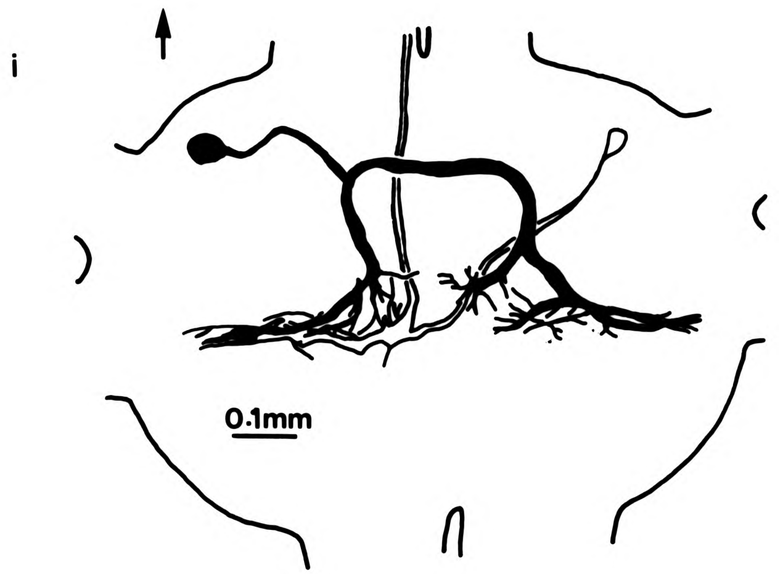
The hypothesis which formed the basis of these double electrode experiments was that the inhibitory response of ANA to low frequencies was mediated by either the ipsilateral or contralateral ON1. In these experiments the terms ipsilateral and contralateral refer to the position of the dendritic area of the omega neurone, on the same side as its cell body, in relation to the dendritic area of ANA. For example, Fig. 3.31(i) shows ANA together with the ipsilateral omega neurone: the dendritic area of the omega neurone on its cell body is ipsilateral to the dendritic area of ANA. Fig. 3.31(ii) shows ANA together with the contralateral ON1: in this preparation the dendritic area on the cell body side of the omega neurone

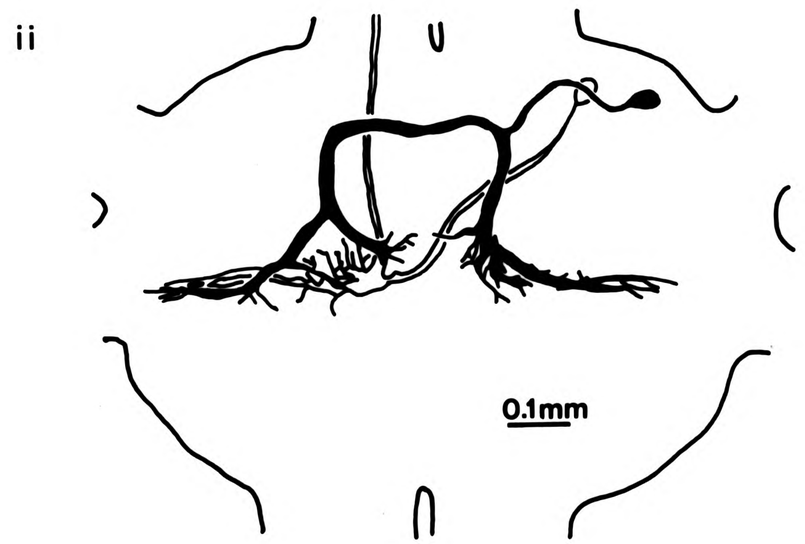
Fig. 3.31.

Camera lucida drawings of ANA and ON1 to show the relative location of these cells in the Pro-thoracic ganglion.

(i) ANA together with the ipsilateral ON1 i.e. the cell body side, dendritic area of ON1 is ipsilateral to the dendritic area of ANA.

(ii) ANA together with the contralateral ON1 i.e. the cell body side dendritic area of ØN1 is contralateral to the dendritic area of ANA.





is contralateral to the dendritic area of ANA. When the omega neurones are considered individually the area on the same side as the cell body is always refered to as the ipsilateral dendritic field and the opposite area is the contralateral dendritic field.

The omega neurones were considered as the most likely mediators of the inhibition because of information obtained from single electrode experiments and because of the results reported by other workers on the properties of these neurones in related cricket species, Gryllus bimaculatus and Gryllus campestris (Wohlers and Huber 1978). In these two European species the omega neurones were shown to be mutually inhibitory: excitation of one, in response to sound presented to its ipsilateral side, resulted in inhibition of the mirror-image omega mediated via the contralateral dendritic area. Since these neurones show this inhibitory effect on each other they may also be mediating the inhibition shown by other neurones, in particular, ANA.

Anatomical and physiological results from single electrode experiments suggested that the ipsilateral ON1 (Fig 3.31(i)) might be responsible for mediating the inhibition. The anatomical evidence was obtained from an experiment using cobalt chloride filled electrodes in which ANA and the ipsilateral ON1 were stained in the same preparation (Fig 3.32). This shows ANA within the pro-thoracic ganglion together with a stain of the

Fig. 3.32.

A double cobalt chloride stain of ANA and the ipsilateral ON1.

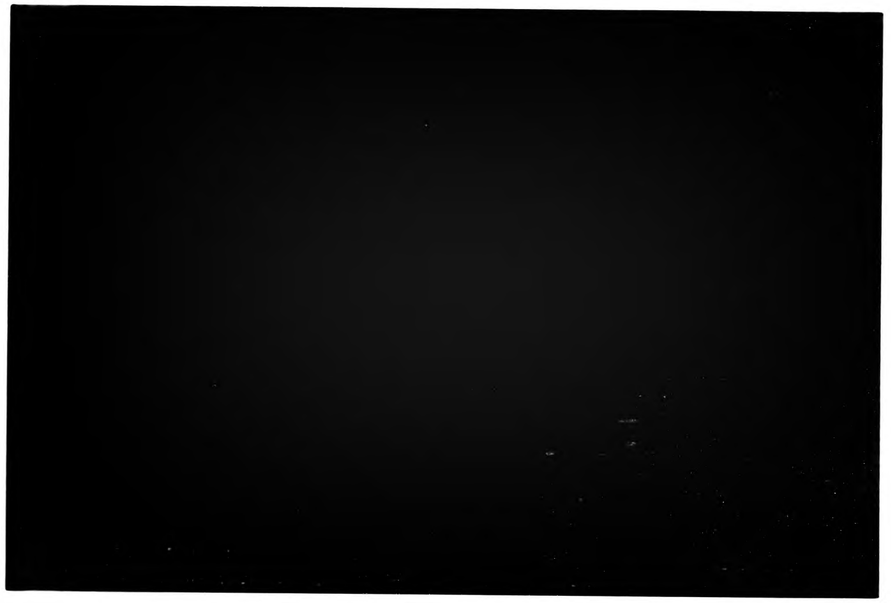
(i) A photograph of the ANA and the contralateral dentritic field of the ON1. The cell body of ON1 and the dendritic area ipsilateral to the cell body were stained faintly

Note: the dendritic areas of the two cells appear to associate across the mid-line of the ganglion.

(ii) A camera lucida drawing of this preparation.

The arrow indicates the anterior direction.

i



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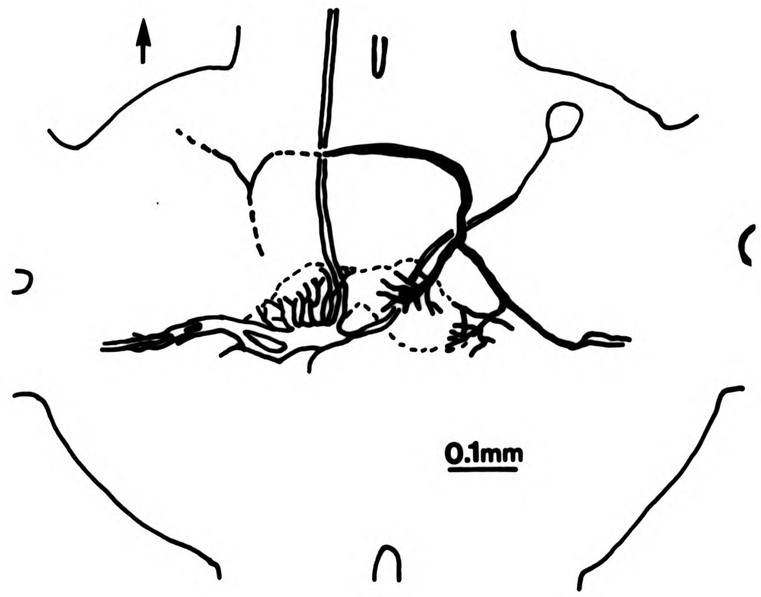


Fig. 3.32.

A double cobalt chloride stain of ANA and the ipsilateral ON1.

(i) A photograph of the ANA and the contralateral dentritic field of the ON1. The cell body of ON1 and the dendritic area ipsilateral to the cell body were stained faintly

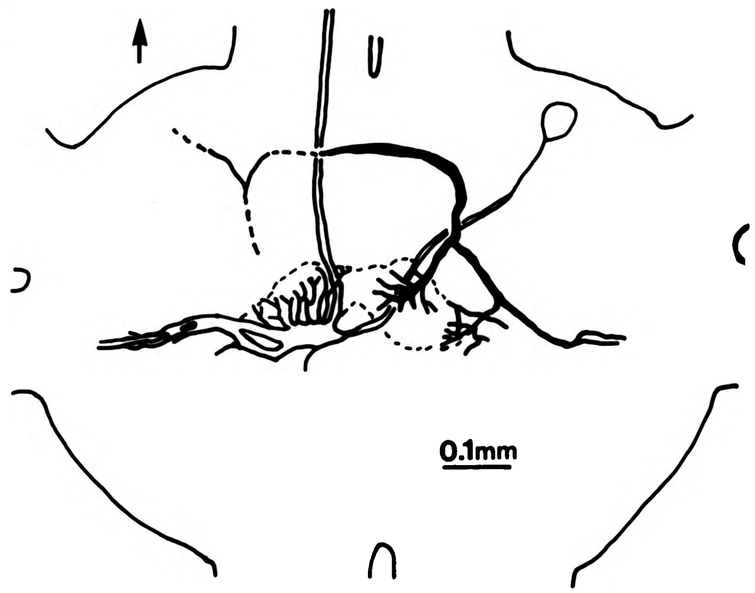
Note: the dendritic areas of the two cells appear to associate across the mid-line of the ganglion.

(ii) A camera lucida drawing of this preparation.

The arrow indicates the anterior direction.

i

ii

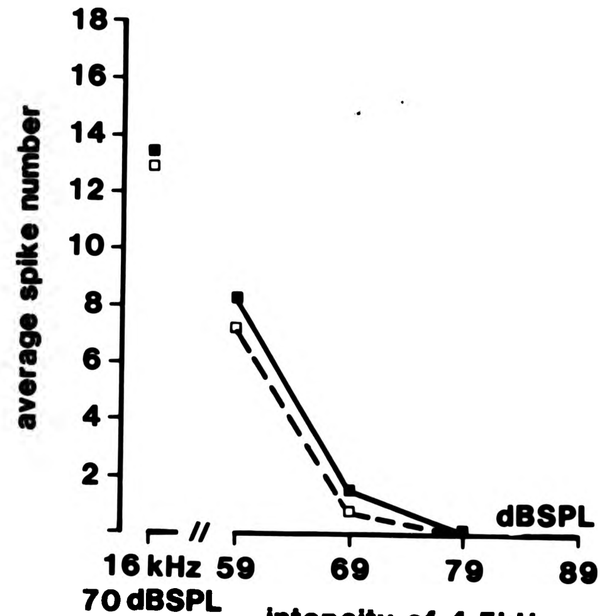


contralateral field of the ipsilateral omega neurone; the cell body and ipsilateral dendritic area of this ON1 were only faintly stained. The large dendritic fibres in the distal part of the dendritic area of ANA cross the axon and project towards the centre of the ganglion where they appear to overlap with the distal fibres of the contralateral dendritic area of ON1. Since the omega contralateral field mediates the inhibition on the mirror-image omega, this could be the site of inhibitory synapses between ANA and the ipsilateral ON1.

Physiological evidence also suggested that the inhibition originated from an ipsilateral neurone. In one experiment ANA's responses to two tone stimuli were recorded before and after the contralateral leg nerve of the preparation was cut close to the ganglion . This prevented any excitatory input to the contralateral side of the ganglion. The average responses over four presentation of each stimulus were calculated and plotted on the graph in Fig 3.33. In the intact preparation the response of ANA to a 16kHz tone was increasingly inhibited by the simultaneous presentation of a 4.5kHz tone at increasing intensities. After the contralateral leg nerve was cut the excitatory response of ANA to the high frequency tone was inhibited, to the same extent, by the presence of the low frequency tone. This result was confirmed in two other preparations in which the whole contralateral leg was cut off at the coxa. Both before and after the amputation ANA

Fig. 3.33.

The effect of cutting the contralateral leg nerve on the inhibitory response of ANA to low frequencies. The first point on each curve represents the response of ANA to 16kHz at 70dB presented alone, the following points represent the response to 16kHz at 70dB plus 4.5kHz at 59dB, 69dB and 79dB in the intact preparation (solid line) and after the leg nerve, contralateral to the dendritic area of the recorded ANA, was cut (broken line).



showed inhibitory responses to low frequency tones.

Experiments to test for the presence of inhibitory connections between the ipsilateral ON1 and ANA (Fig. 3.31(i)) were carried out.

The results from one of the first experiments to investigate the existance of an inhibitory connection between the ipsilateral ON1 and ANA are shown in Fig 3.34. In this preparation the ipsilateral ON1 showed a limited response to frequencies which produced an excitatory response in ANA: 14kHz at 75dBSPL produced a burst of excitation in ANA consisting of 7-8 spikes whereas the response of the omega neurone consisted of only 2 spikes (Fig. 3.34(i)). Also, ON1 showed an excitatory response to frequencies that produced inhibition in ANA: 4kHz at 80dB plus 14kHz at 75dB (Fig. 3.34(ii)), and 4kHz at 80dB alone (Fig. 3.34(iii)) produced an average of 10 spikes in the omega neurone but resulted in no spiking in ANA. These results supported the suggestion that the ipsilateral ON1 mediates the inhibition on ANA.

However, as later experiments revealed, this result was atypical. The threshold curves in Fig 3.35 were taken from ANA and the ipsilateral ON1 in the same preparation. At frequencies above 8kHz the threshold levels of ANA and ON1 are very similar: the threshold level at 18kHz was 38dBSPL in both neurones. At 5kHz the neurones show the maximum difference in threshold levels: 33dBSPL was the

Fig. 3.34.

Simultaneous recordings of the extracellular response of ANA and the intracellular responses of the ipsilateral ON1 neurone to,

(i) 14kHz at 75dB

(ii) 14kHz at 75dB plus 4kHz at 80dB

(iii) 4kHz at 80dB

Upper trace represents the extracellular response of ANA. Middle trace represents the intracellular response of ON1. The deflection of the lower trace represents the stimulus.

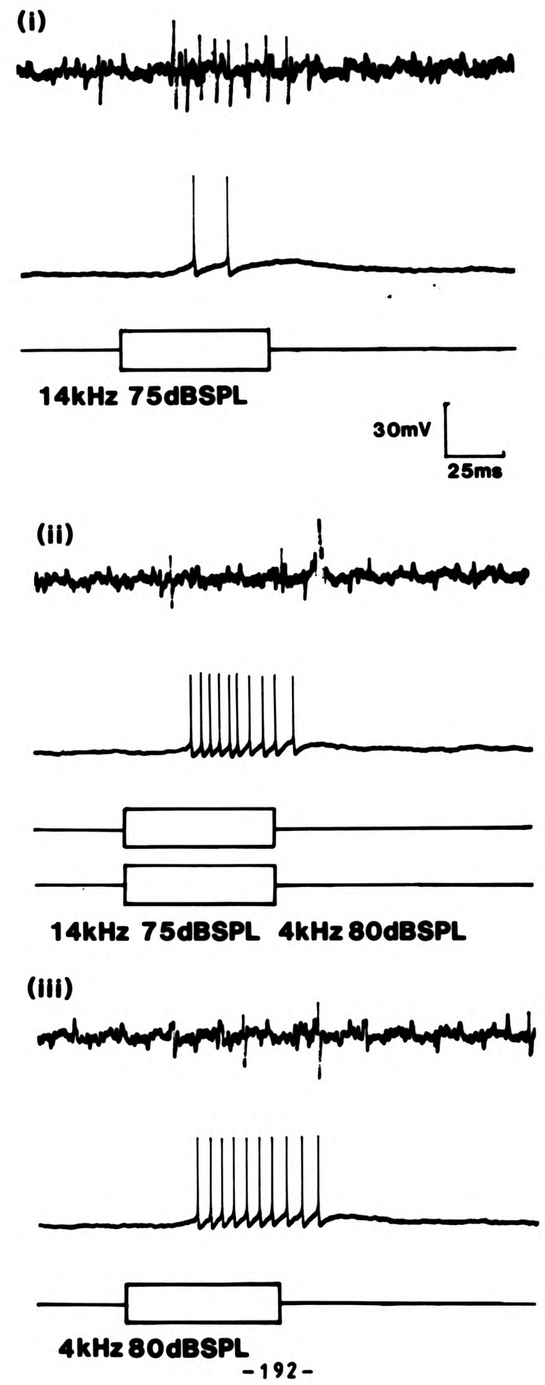
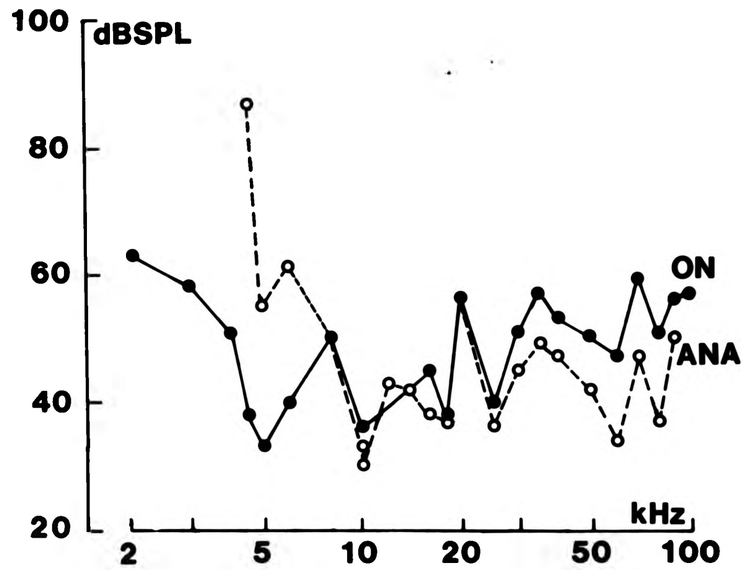


Fig. 3.35.

Threshold curves from ANA and ON1 showing the relative sensitivity of each neurone to a range of frequencies. The solid line represents the threshold curve of ON1 and the broken line represents the threshold curve of ANA.
Note: Both threshold curves were taken from neurones in the same preparation.



threshold level of the response in ON1 to 5kHz compared with 55dBSPL for ANA at this frequency. ON1, therefore shows a similar sensitivity of response to low and high frequency tones whereas although the sensitivity of ANA to low frequencies is very low its sensitivity to high frequencies is comparable with that of ON1

The supra-threshold responses of ON1 and ANA to two tone stimuli were considered in more detail by testing them, in the same preparation, with a range of intensities of the low frequency tone. The number of spikes shown by both neurones in response to each intensity were counted and plotted as shown in Fig 3.36. The first point on each curve represents the number of spikes in response to a single 14kHz tone at 73dBSPL. The points following this represent the neurone's responses to the same tone presented together with a 4.5kHz tone over a range of intensities. The simultaneous presentation of 4.5kHz at intensities above 49dBSPL produced inhibition of the excitatory response of ANA to 16kHz. Initially, low intensities of 4.5kHz produced an increase in the responses of ON1 to 16kHz. However, above 49dBSPL further increases in the intensity of the 4.5kHz tone resulted in a decrease in ON1's response. Single traces of the responses of ON1 in this preparation to high intensities of 4.5kHz alone are shown in Fig. 3.36.1. 4.5kHz at 64dBSPL produced 19 spikes but a 10dB increase in intensity reduced this to 13 spikes. A further increase in intensity to 84dBSPL produced 12

Fig. 3.36.

Simultaneous recordings of the extracellular response of ANA and the intracellular response of ON1 to 14kHz at 73dB plus 4.5kHz presented at 39dB, 49dB, 59dB, 69dB and 79dB. The first point on each graph represents the response to 14kHz at 73dB alone. The subsequent points represent the response of ANA (solid line) and ON1 (broken line) to the two tone stimuli.

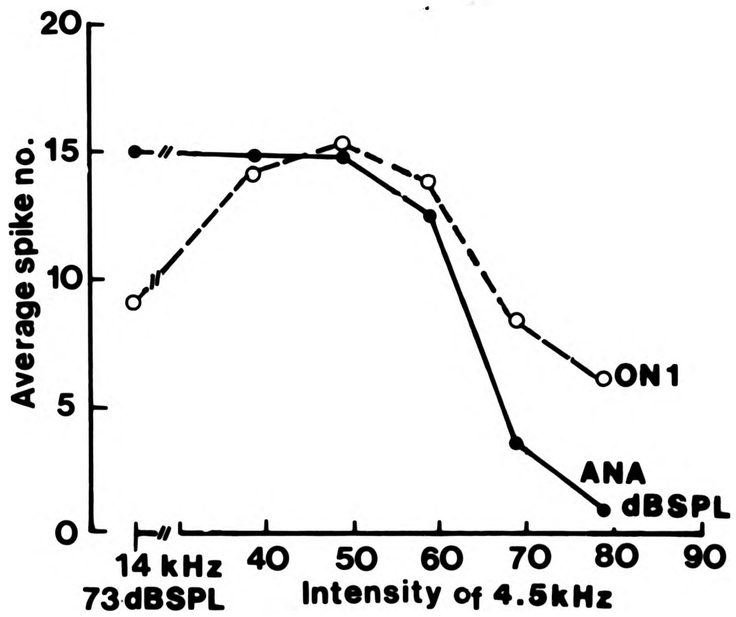
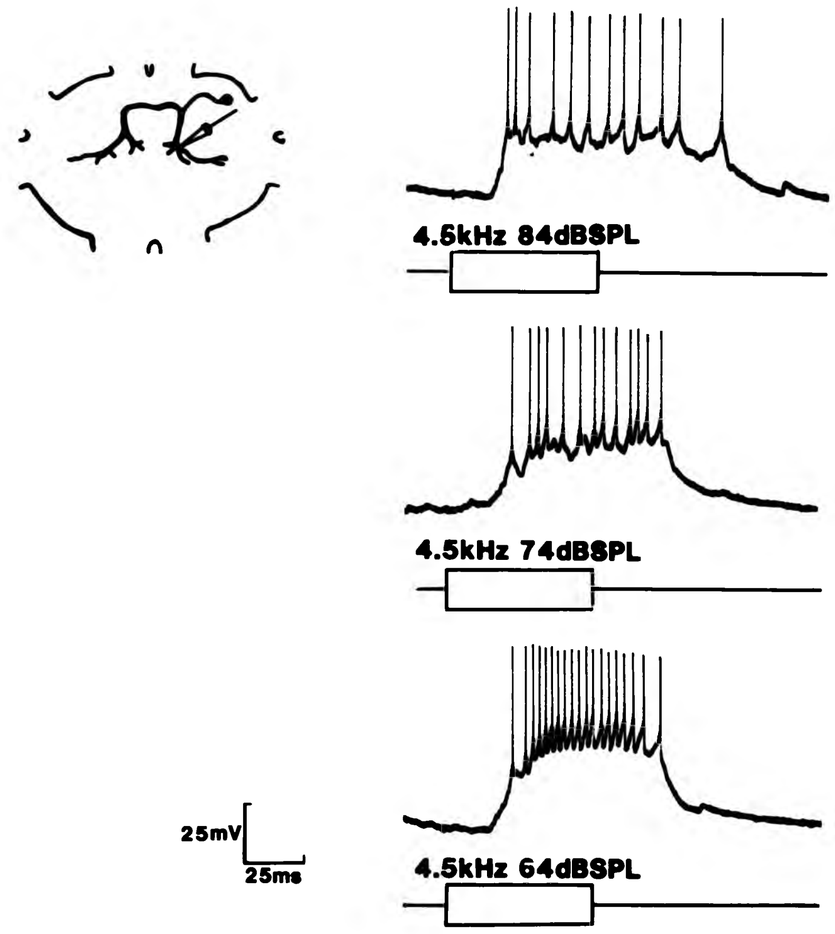


Fig. 3.36.1

Intracellular recording of tne response of ON1 to 4.5kHz presented at 64dB, 74dB and 84dB.

Note that the responses decrease at high intensities.



spikes and an increase in the duration of the response. The results of single electrode experiments on ON1 with the application of picrotoxin (Fig 3.21) showed that this reduction in response was due to the presence of a high intensity inhibitory input. Therefore intensities of the low frequency tone which produce inhibition in ANA also inhibit the response of ON1, to a limited extent.

3.5.1. Current manipulation of the ipsilateral ON1.

Current manipulation experiments, using the double electrode recording set-up, were carried out in ten preparations to investigate, electrophysiologically, the existence of synaptic connections between ANA and the ipsilateral ON1. The results from one preparation, in which hyperpolarising current was used to manipulate the responses of ON1 during two tone stimulation are shown in Fig 3.37. 14kHz at 73dBSPL together with 4.5kHz at 89dBSPL produced 7 spikes in the ipsilateral ON1 and complete inhibition in ANA. The injection of 5nA of hyperpolarising current completely suppressed the response of ON1 to these frequencies but produced no release of inhibition in the response of ANA. A similar result was shown when the neurones' responses were tested over a range of intensities of the 4.5kHz tone. The graph in Fig. 3.37(ii) represents the average spike number shown by ANA in response to 14kHz at 73dBSPL together with 4.5kHz at intensities between 49dBSPL and 89dBSPL with and without

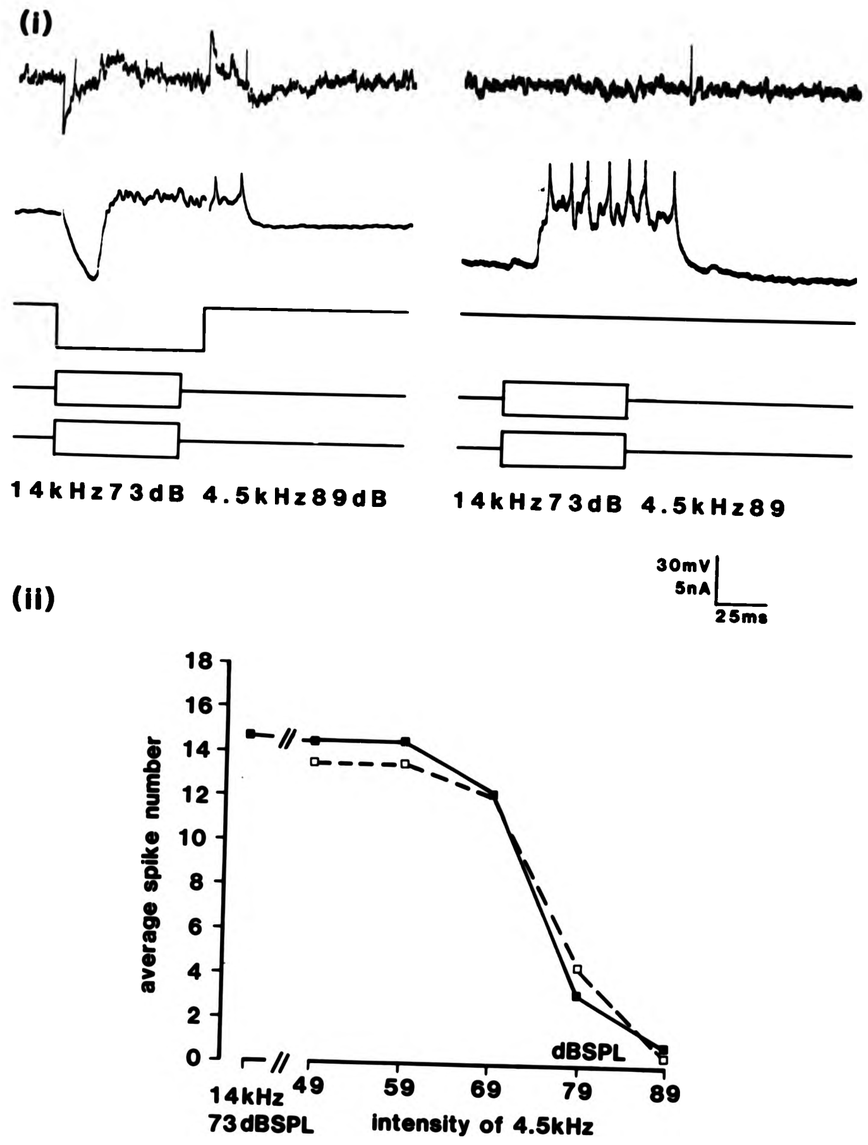
Fig. 3.37.

Simultaneous recordings of the extracellular responses (upper trace) of ANA and the intracellular responses (second trace) of the ipsilateral ON1 with manipulation of the ON1 activity using hyperpolarising current (third trace). The two lower traces represent the tone stimuli.

(i) Extracellular responses of ANA and intracellular responses of ON1 to 14kHz at 73dB plus 4.5kHz at 89dB alone (right) and together with 5nA of hyperpolarising current (left).

(ii) Intensity/response curves of ANA to 14kHz at 73dB plus 4.5kHz at 49dB, 59dB, 69dB, 79dB and 89dB with no current manipulation of the response of ON1 (solid line) and with suppression of the response of ON1 using hyperpolarising current (broken line).

Note: Manipulation of the response of ON1 has no following effect on the response of ANA.



the presence of complete suppression of the ipsilateral ON1's response by the injection of 5nA of hyperpolarising current. Clearly, suppression of the response in ON1 had no effect upon the response of ANA to any of the intensities tested.

If synaptic connections were present between the ipsilateral ON1 and ANA, increasing the response of ON1 to a high frequency tone by injecting depolarising current should initiate inhibition in the response of ANA to this tone. The traces in Fig. 3.38(i) show that the response of ON1 to 14kHz at 53dBSPL was increased from 4 spikes to 12 spikes by the injection of 2nA of pulsed, depolarising current but this did not produce a decrease in the response of ANA which remained at 9 spikes. The graph in part (ii) confirms this result over a range of intensities of 14kHz: it represents the response of ANA before and after the injection of depolarising current to increase the response of ON1.

Therefore, to summarise, manipulation of the ipsilateral ON1 response, by injection of either depolarising or hyperpolarising current, produces no corresponding change in the response of ANA. This suggests that there are no effective synaptic connections between these two neurones.

3.5.2. Current manipulation of the contralateral ON1.

Mutual inhibition between the paired ON1 neurones is

Fig. 3.38.

Simultaneous recordings of the extracellular responses of ANA (upper trace) and the intracellular responses of the ipsilateral ON1 (lower trace) with manipulation of the ON1 response using depolarising current (third trace). The lower trace represents the tone stimulus.

(i) Extracellular response of ANA and intracellular response of ON1 to 14kHz at 53dB alone and together with 2nA of depolarising current.

(ii) Intensity response curves of ANA to 14kHz presented at 43dB, 53dB, 63dB, 73dB and 83dB with no current manipulation of the response of ON1 (solid line) and with an increase in the response of ON1 (broken line) by the injection of depolarising current.

Note: Manipulation of the response of the ipsilateral ON1 has no following effect on the response of ANA.

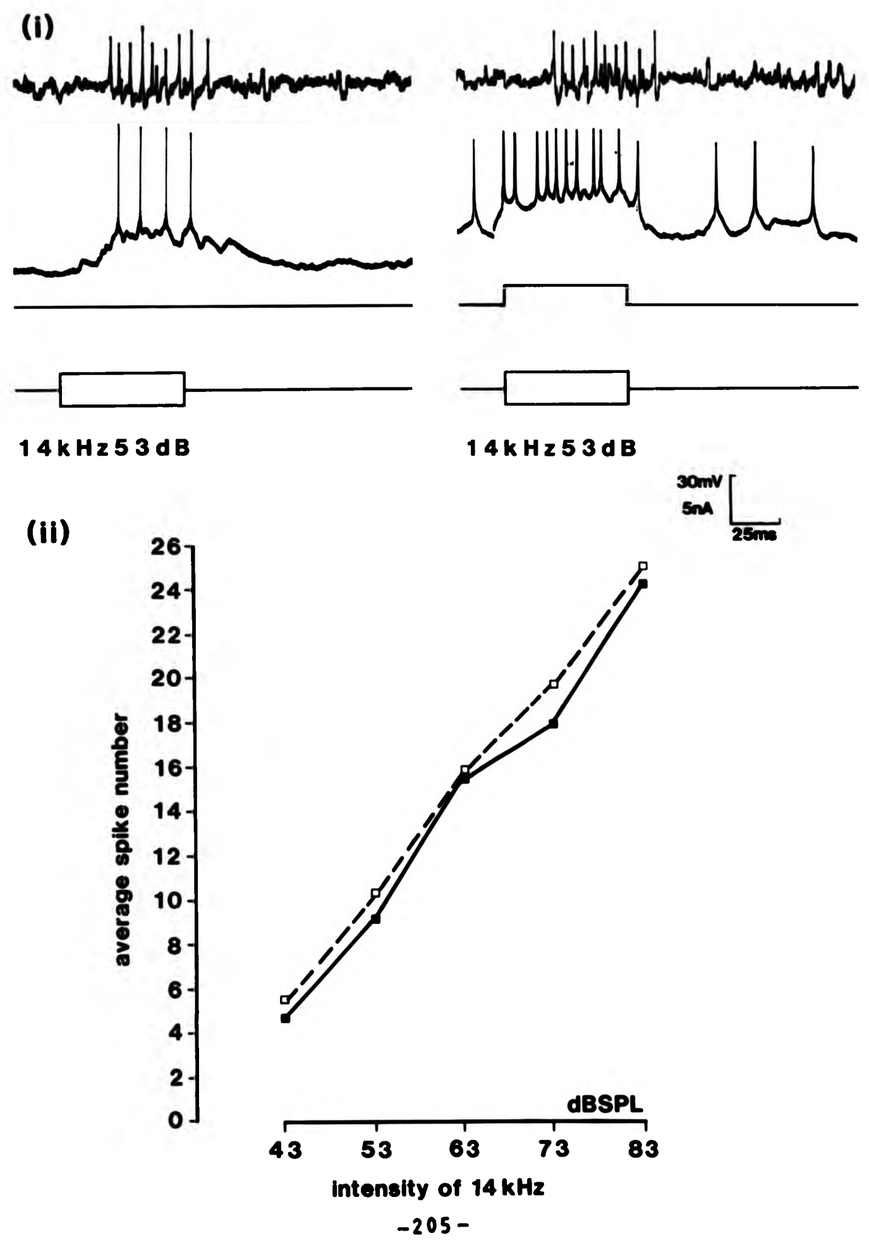


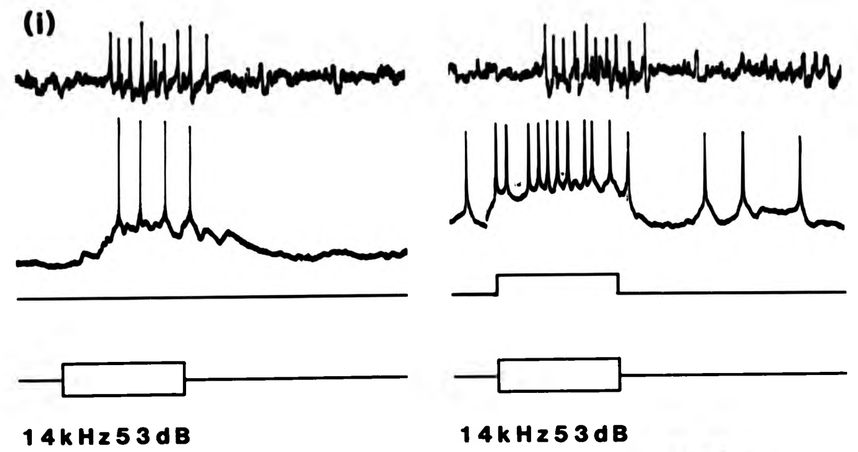
Fig. 3.38.

Simultaneous recordings of the extracellular responses of ANA (upper trace) and the intracellular responses of the ipsilateral ON1 (lower trace) with manipulation of the ON1 response using depolarising current (third trace). The lower trace represents the tone stimulus.

(i) Extracellular response of ANA and intracellular response of ON1 to 14kHz at 53dB alone and together with 2nA of depolarising current.

(ii) Intensity response curves of ANA to 14kHz presented at 43dB. 53dB, 63dB, 73dB and 83dB with no current manipulation of the response of ON1 (solid line) and with an increase in the response of ON1 (broken line) by the injection of depolarising current.

Note: Manipulation of the response of the ipsilateral ON1 has no following effect on the response of ANA.



mediated via the contralateral dendritic areas. In the contralateral omega neurone this area overlaps with the dendritic area of ANA (Fig. 3.31(ii)) this therefore, may be the source of the inhibition on ANA. To test this hypothesis a series of experiments involving simultaneous recordings from the contralateral omega neurone and ANA were carried out.

Current manipulation experiments were carried out on the contralateral ON1 and ANA in five preparations. In each preparation, after the test protocol was complete, the contralateral omega neurone was anatomically identified by the injection of Lucifer Yellow CH. Similar results were obtained from each preparation. Fig 3.39 shows the responses from one of the preparations in which hyperpolarising current was used to suppress the response of ON1. 14kHz at 73dBSPL presented simultaneously with 4.5kHz at 89dBSPL produced an excitatory response of 16 spikes in ON1 and complete inhibition of the response in ANA. 5nA of hyperpolarising current injected into the omega neurone reduced its response to 4 spikes; however, ANA still showed complete inhibition. ANA shows this same response over a range of 4.5kHz intensities. This is illustrated by the graph in Fig. 3.39(ii) which shows the responses of ANA to these frequencies before and after the responses of the contralateral omega were suppressed with hyperpolarising current. To complete the experiment, the effect of depolarising current on the responses of both

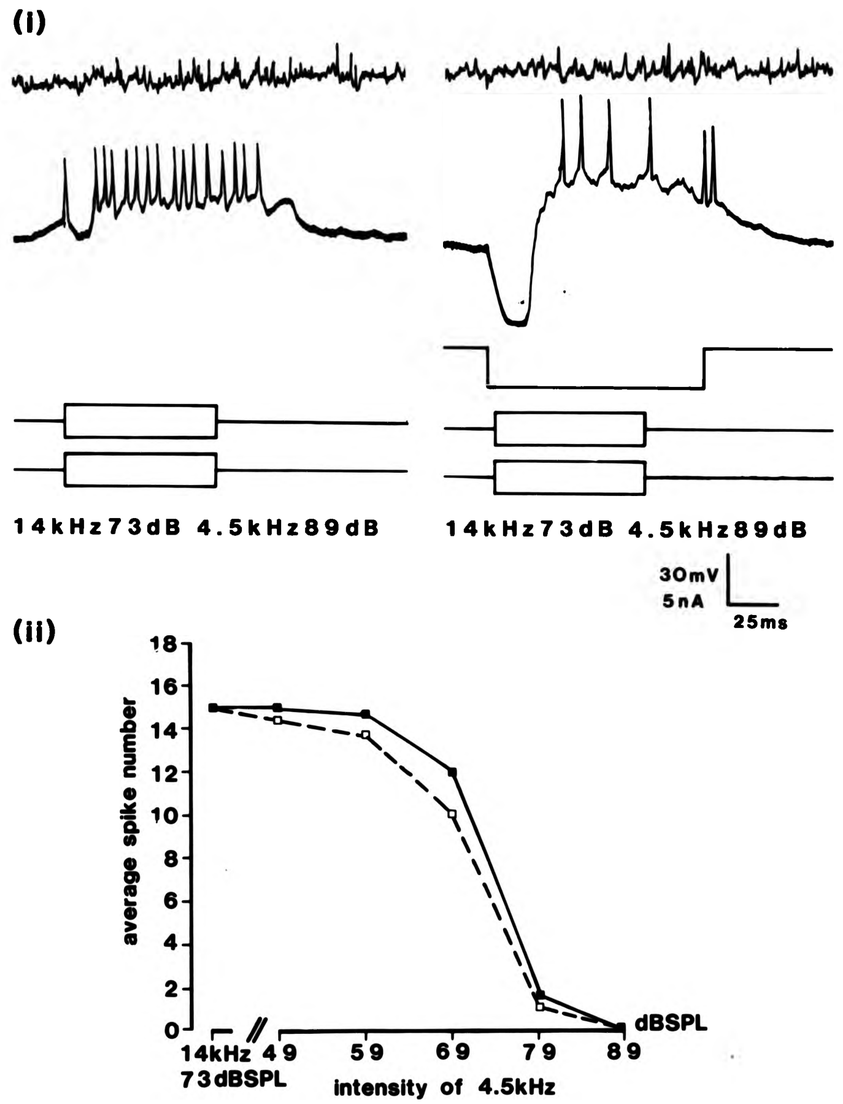
Fig. 3.39.

Simultaneous recordings of the extracellular responses of ANA (upper trace) and the intracellular responses of the contralateral ON1 (second trace) with manipulation of the response of ON1 using hyperpolarising current (third trace). The two lower traces represent the tone stimuli.

(i) Extracellular response of ANA and intracellular response of ON1 to 14kHz at 73dB plus 4.5kHz at 89dB alone, and together with 5nA of hyperpolarising current.

(ii) Intensity response curves of ANA to 14kHz at 73dB plus 4.5kHz presented at 49dB, 59dB, 69dB, 79dB and 89dB with no current manipulation of the response of ON1 (solid line) and with suppression of the response of ON1 (broken line) by the injection of hyperpolarising current.

Note: Manipulation of the response of the contralateral ON1 has no following effect on the response of ANA.



neurones was tested. The results are shown in Fig. 3.40. The traces in Fig. 3.40(i) show that while the injection of 2nA of depolarising current produced an increase in the response of ON1 from 11 spikes to 20 spikes, this failed to initiate an inhibitory response in ANA even over the range of intensities of 14kHz shown in Fig 3.40(ii).

In one preparation this type of current manipulation experiment was carried out on both the ipsilateral and contralateral ON1 neurones. In both cases hyperpolarising current completely suppressed the responses of the omega neurones but there was no release of inhibition in the ANA.

Therefore, these current manipulation experiments have shown that neither the ipsilateral nor the contralateral ON1 neurones are responsible for mediating the inhibition shown by ANA in response to high intensities of low frequency tones.

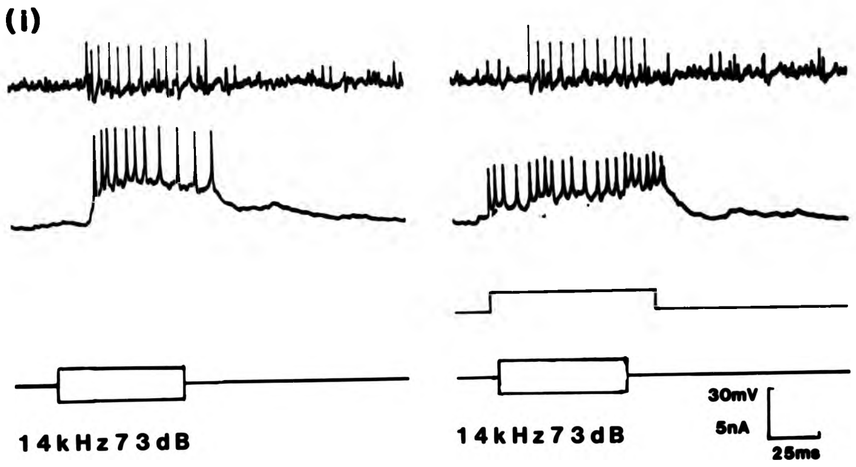
Fig. 3.40.

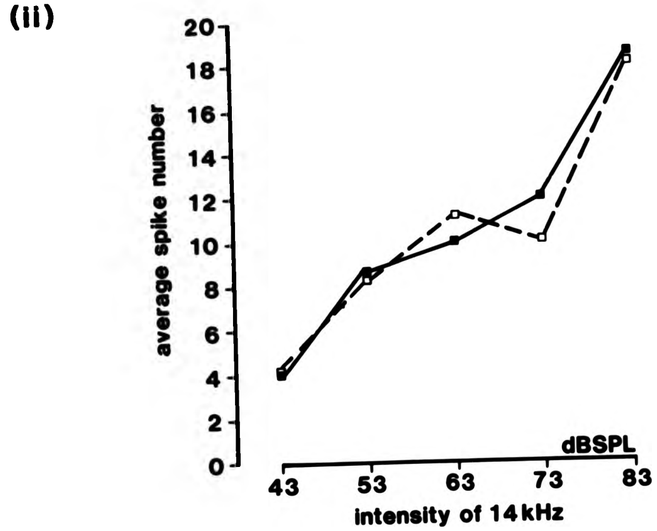
Simultaneous recordings of the extracellular responses of ANA (upper traces) and the intracellular responses of the contralateral ON1 (second trace) with manipulation of the response of ON1 using depolarising current (third trace).

(i) Extracellular response of ANA and intracellular response of ON1 to 14kHz presented at 73dB alone (left) and together with 2nA of depolarising current (right).

(ii) Intensity response curves of ANA to 14kHz presented at 43dB, 53dB, 63dB, 73dB and 83dB with no current manipulation of the response of ON1 (solid line) and with an increase in the response of ON1 (broken line) by injection of depolarising current.

Note: Manipulation of the response of the contralateral ON1 has no following effect on the responses of ANA.





CHAPTER 4.

DISCUSSION.

For conspecific songs to be effective in releasing the appropriate behaviour individuals within the species must be able to recognise and distinguish between the different song types. The parameters available for this depend, to some extent, on the species. Analysis of the frequency spectra of the songs of Gryllus campestris, a European species of field cricket, shows that both the calling and aggression songs contain a characteristic carrier frequency at around 4.5kHz and a secondary peak (equivalent to the third harmonic) at around 15kHz which is 20-25dBSPL less intense than the main peak. In contrast, the spectrum of the courtship song shows its maximum peak near 15kHz which is at least 40dBSPL more intense than the 4kHz region (Nocke 1972). The calling and aggression songs have species specific temporal patterns of syllables which are grouped into chirps. The courtship song consists of repeated "ticks". Since the frequency components of the calling and aggression songs are the same they could be distinguished on the basis of temporal characteristics. Recognition of the courtship song however can be based entirely on frequency.

Spectrograms of the songs of T. oceanicus show that all three contain similar frequencies. Each has a carrier frequency of around 4.5-5kHz and higher harmonics from 10kHz to 55kHz (Latimer and Lewis 1986). The secondary peak at around 10kHz is 10-15dB down on the intensity of the carrier frequency. Because the frequencies are similar in

the three songs the most obvious parameter for song type recognition is the temporal pattern. Each song, in particular the courtship song, has a distinctive, highly complex temporal pattern in which the syllables are arranged in both chirps and trills (Bentley and Hoy 1972). Interneurones capable of producing a synchronised response that accurately reproduced these temporal patterns must form the basis of recognition of these songs. In this project investigations into the physiological and morphological properties of the ventral nerve cord neurones were carried out on the basis of the hypothesis that these neurones, by producing a synchronised response to all or part of the temporal structure of the song, were involved in the process by which crickets recognise and distinguish between their conspecific songs.

The results suggest that ANC is particularly involved in calling song recognition.

4.1. The role of ANC in Calling Song Recognition.

ANC (Fig. 3.1) is a low frequency neurone with a best frequency response at 5kHz (Fig. 3.2) which corresponds to the carrier frequency of all three song types. In response to a pre-recorded tape containing examples of the natural songs, ANC produced spikes correlated with the temporal structure of the syllables in the calling and aggression songs but only with the chirp phrase of the courtship song (Fig. 3.4): the rapid

syllable rate of the trill phrase of this song produced a burst of excitation in ANC in which it was not possible to discerne the unit's response to individual sylables. The temporal structure of the trill phrase is the only difference between the calling and courtship songs. Their recognition therefore, depends upon accurate interpretation of the temporal patterns of the trill phrases. The synchronised response ANC produced to the calling song suggests it may be involved in the neural pathway responsible for calling song recognition.

The main function of the calling song is to attract the female in preparation for courtship. Therefore as she approaches the singing male the female has to code the song pattern over a considerable range of intensities. ANC is particularly sensitive to 5kHz and so is able to respond to low intensities of the song when the male is singing at a distance. In addition, ANC is able to copy the temporal pattern at high intensities (when the song is produced at close range), because it exhibits a limited amount of two tone suppression which produces high intensity inhibition in its response to 4-5kHz (Fig.3.3). This inhibition prevents saturation of ANC's response to high intensities of this frequency which, in the context of the song, would allow the neurone to maintain accurate coding even at very high intensities.

This is the first description of ANC in T. oceanicus but it has been suggested that homologous neurones,

described in other species, also have a role in calling song recognition. A unit described in Gryllus camperstis as AN1 (Wohlers and Huber 1982) shows similar physiological and morphological characteristics and may therefore be homologous to ANC. AN1 has a best frequency response at 4-5kHz and shows a correlated response to syllables representing the temporal structure of the calling song. The "pulse-coder", recorded by Stout and Huber (1972, 1981) in G. campestris, is probably the same cell as AN1. A second type of low frequency ascending neurone (AN3) has been detailed in G. campestris. (Boyd, Kuhne, Silver and Lewis 1984). Although both AN1 and AN3 have not been identified in the same preparation they have characteristic anatomical differences which are consistant with them being separate neurones. The dendritic areas in AN1 and ANC consist of a dense bundle of fibres situated towards the centre of the ganglion. In contrast, AN3 has an additional dendritic branch which projects from the centre out towards the leg nerve. AN3 has inhibitory side-bands at 3kHz and 16kHz which tune the neurone's responses to 5kHz (Boyd et al. 1984). AN3 is more sensitive at this frequency than AN1 therefore it's suggested role is in coding for the song when it is presented at low intensities over long distances. The same function has been suggested for a low frequency neurone, PALF1, recorded from its projection in the supra-oesophageal ganglion (brain) of G. bimaculatus (Boyan and Williams 1982). This neurone showed a low

response threshold to 5kHz but a rapid saturation of its response at this frequency with increased intensities. LF1, a low frequency ascending neurone, recorded from the prothoracic ganglion of the same species (Rheinlaender, Kalmring, Popov and Rehbein 1976) is thought to be the same neurone as PALF1. LI, in Acheta domesticus. (Stout, Atkins and Burghardt 1985) has a dendritic branch which projects out towards the leg nerve. It may therefore represent a homologue of AN3 in this species. Extracellular recordings from the cervical connectives of T. commodus, a congeneric species of T. oceanicus, showed the presence of a low frequency tuned ascending neurone but as no anatomical details were given it is not possible to determine if this is homologous to ANC or AN3 (Ball and Hill 1978; Boyan 1979). To date, no homologue for AN3 has been found in T. oceanicus. Since, in T. oceanicus, ANC can code over a wide range of calling song intensities, a second low frequency neurone tuned to low intensities of the calling song may be unnecessary in this species. The results therefore suggest that ANC's primary function may be in recognition of the calling song.

Compared to calling song recognition, investigations into the mechanisms by which crickets recognise their courtship songs are limited. The main reason for this may be is that the majority of studies have been carried out on cricket species in which the courtship song has a different carrier frequency from the calling song.

Recognition of the song types in these species can therefore be based on neurones tuned to different frequencies. In T. oceanicus the situation is different in that all three of the song types have the same frequency spectra and therefore the same carrier frequency. In this species it is likely that recognition is based on temporal pattern differences between the songs rather than frequency differences. Double cobalt chloride stains showed that ANC coexists in the pro-thoracic ganglion with the other ascending neurone ANA and results from both extracellular and intracellular experiments strongly suggest that ANA, by producing an accurate representation of the temporal pattern of the trill phrase of the courtship song, is primarily involved in courtship song recognition.

4.2. The role of ANA in courtship song recognition.

The extracellular experiments carried out on ANA (Fig. 3.5) confirmed results previously shown for this neurone (Hutchings and Lewis 1984). Physiologically, ANA is a broad band neurone which responds with varing sensitivity to frequencies between 4kHz and 100kHz, has a best frequency at around 12-16kHz (Fig. 3.7) and shows strong inhibition in response to low frequencies (Fig. 3.8) particularly at high intensities (Fig. 3.9). An ascending neurone, Int-1 (Casaday and Hoy 1977), also recorded from T. oceanicus (Moiseff and Hoy 1983), is likely to be the same cell as ANA. Morphologically the two cells are very

similar. However, there are some differences in their physiological characteristics. Like ANA, Int-1 showed two-tone suppression (Moiseff and Hoy 1983) but the extent of this suppression was far less than that of ANA. In addition, at very high intensities of a low frequency tone Int-1 showed an increase in excitation compared to its response to lower intensities (Nolen and Hoy 1986b). These differences are difficult to explain. In G. bimaculatus a high frequency, ascending neurone termed HF1AN (Popov and Markovich 1982) shows similar physiological and morphological characteristics to ANA and therefore it seems likely that this neurone is homologous to ANA. The high frequency ascending neurone, PAHF1, recorded from its terminal arborisations in the brain of G. bimaculatus (Boyan and Williams 1982), has the physiological characteristics of HF1AN which suggests they are the same cell. Although the dendritic arborisations of PAHF1 in the supra-oesophageal ganglion are comparable with those of ANA (Fig. 3.6), PAHF1 typically, has a looped dendritic projection in the optic stalk which is not present in ANA. HF1, also recorded in G. bimaculatus (Rheinlaender, et al. 1976) is physiologically similar to PAHF1 but, like ANA has no dendritic area in the optic lobe. Therefore, HF1 and PAHF1 may represent two separate high frequency ascending neurones in G. bimaculatus. In G. campestris Wohlers and Huber (1978) recorded an Auditory Interneurone with an Ascending Axon (AIAA) which showed a varying response to

4.5kHz but a strong excitatory response to frequencies above 10kHz. This neurone was renamed AN2 (Wohlers and Huber 1982) and is probably homologous to ANA. The homologue of ANA in Acheta domesticus is likely to be L2, a high frequency neurone which is anatomically similar to ANA (Stout et al. 1985).

It has been sugested that each of these neurones, because they show an excitatory response to high carrier frequencies may have a role in courtship song recognition and predator detection. The exception is Int-1 in T. oceanicus. The carrier frequency of the courtship song in this species is around 4.5kHz. In most other cricket species the carrier frequency of the courtship song is around 15kHz. Because Int-1 is inhibited by low frequencies but shows strong excitation to high frequencies (Moiseff and Hoy 1983) it has been suggested that this neurone's main function is in predator detection (Nolen and Hoy 1986a). Crickets (like moths; Roeder 1967) are known to fly at night, which may render them susceptable to bat predation. In flight (Moiseff, Pollack and Hoy 1978, Nolen and Hoy 1986a) and during walking (Pollack, Huber and Weber 1984), crickets also show a sterotyped avoidance behaviour in the presence of ultrasonic frequencies in the range of bat echolocation signals. This behaviour is called negative phonotaxis and involves the insect actively turning away from the direction of the sound source. If the activity of Int-1 in response to ultrasonic stimuli is suppressed

during flight, by injection of hyperpolarising current, the negative phonotaxis is abolished (Nolen and Hoy 1984) indicating that this neurone is involved in the behaviour. The high frequency neurone in G. bimaculatus, HF1AN, responds to simulations of ultrasonic bat cries (Popov Øand Markovich 1982) but manipulation of its activity in the presence of the behavioural response has not been tested. The extent to which crickets are preyed upon by bats is not clear. Popov and Shuvalov (1977) observed G. bimaculatus flying in the same location as hunting bats. The crickets were seen to fall to the ground when a bat was at close range which is similar to the evasive behaviour (passive nose-dives) shown by lacewings when being pursued by bats (Miller and Olesen 1979; Miller 1983).

To initiate avoidance behaviour in flight, activity in Int-1 must exceed 220 spikes/second (Nolen and Hoy 1984). However, during walking this activity in Int-1 alone is insufficient to produce negative phonotaxis. The results of experiments carried out in this project suggest that, despite the fact that ANA is inhibited by low frequencies and the carrier frequency of the courtship song is 4.5kHz, in common with its homologous neurones in other species, ANA's main function is in courtship song recognition. Because low frequencies produce inhibiton in ANA, its role in courtship song recognition does not conflict with the proposed role of Int-1 in predator detection (this is discussed in more detail in section 4.4)

Extracellular experiments (Fig. 3.10) confirmed ANA's ability to respond to each of the natural song types and to simulations of the courtship song containing high and low frequencies (Hutchings and Lewis 1984). To investigate the mechanisms by which ANA produces a response to songs which have a carrier frequency of 4.5kHz a series of intracellular experiments where carried out.

50ms test pulses presented at a rate of 2/s and containing high intensities of a 4.5kHz tone presented alone and together with a 16kHz tone commonly produced inhibition in ANA. This response consisted of an initial complex IPSP response followed by repolarisation, and a small depolarising overshoot before returning to resting level (Fig. 3.12). Increasing the duration of the stimulus changed this response. A 160ms duration pulse produced an increase in the amplitude of the depolarising phase so that it was raised beyond threshold and produced a spike. There was no corresponding increase in either the amplitude or duration of the hyperpolarising phase (Fig. 3.12.1). It is therefore unlikely that the increase in the amplitude of the depolarisation is the result of a rebound effect from enhanced inhibition. It is more likely to be the result of an underlying, longer duration excitatory response. This is indicated by ANA's response to low intensities of low frequency tones in which the inhibition is replaced by a small amount of excitation (Fig. 3.13) and was confirmed by the results of experiments in which Picrotoxin was used to

block the inhibitory response of ANA to a low frequency tone. This showed the existance of an underlying excitation, usually masked at high intensities by strong inhibition (Fig. 3.14). Picrotoxin mediates its effect by blocking chloride channels and therefore preventing the initiation of IPSPs (Cooper et al. 1982). The early work of Suga and Katsuki (1961) on T fibres (McKay 1969) in grasshoppers showed that picrotoxin was effective at insect inhibitory synapses. More recent work involving intracellular experiments showed application of Picrotoxin prevented locust auditory neurones from habituating in response to repeated stimuli (Romer and Seikowski 1985).

It is the integration of the excitatory response with the inhibitory response which allows ANA to produce an accurate code of the courtship song. Detailed analysis of ANA's response to simulations of the courtship song containing 4kHz at high intensity (87dB) showed that the presence of single spikes, synchronised to each syllable of the trill phrase, are the result of summation of the hyperpolarising and depolarising phases of the responses to successive syllables (Fig. 3.15.1). The syllable rate of the initial chirp phrase allows this summation of response which leads to an elevation of the overall potential which ultimately reaches spike threshold. The rapid syllable rate in the subsequent trill phrase maintains this potential level and results in a response of one spike per syllable. ANA is therefore able to code for the courtship song

because of the presence of an underlying excitatory input in combination with an inhibitory input. The excitatory input produces the depolarising phase of the response, and: the inhibitory input limits the extent of the excitation, maintaining accurate coding.

This explanation is based on the response of ANA to simulations of the courtship song containing 4kHz (87dB) alone. To produce a more accurate simulation of the song, 4.5kHz (89dB) was presented simultaneously with 16kHz (70dB). This represents the correct relative intensities of these frequencies in the natural song (Hutchings and Lewis 1984). Because of the high intensity inhibition shown by ANA in response to low frequencies this simulation also produced an accurately coded response in ANA. However the simulations of the calling and aggression songs, containing the same frequencies produced very little response in ANA. The calling and aggression songs differ from the courtship song in two ways: the syllable rate of the trill phrase of the courtship song is faster than the syllable rates of any part of the calling or aggression songs: the intensity of the courtship trill is 6dB below that of the chirp phrase of this song and of both the calling and aggression songs. This suggests that the ability of ANA to produce an accurate code of the courtship song is based on the syllable rate of this song and the intensity at which it is received.

4.3. The importance of syllable rate and intensity in song recognition.

It is generally considered that the role of the ascending neurones is to produce a "template" of incoming sounds which is then conveyed to the brain where it is "recognised". To date, there is no evidence that any of the ascending neurones are specifically tuned to the temporal patterns of the songs. The results presented here however, suggest that ANA acts to some extent as a temporal filter for the courtship song. The importance of syllable rate was confirmed by testing the response of ANA to slower trill rates. This results in a less accurate response by ANA to the syllable pattern (Fig. 3.16). The effect of intensity was also investigated. The accuracy of ANA's response to simulations of the courtship trill phrase presented at different intensities was compared. The response is predictably poor when presented at low (near threshold) intensities (58dB). Above this, ANA produces an accurate correlation to the syllable rate; however, at very high intensities the correlation is poor (Fig. 3.16.1). It appears that at these high intensities a greater inhibition occurs in ANA, with the result that at fast repetition rates the response to the second syllable is imposed on a potential which is still below threshold so there is very little spiking. Although this appears surprising the amplitude changes in the natural song result in the trill phrase being 6dB less intense than the chirp phrase which

may represent an adaptation to prevent this over inhibition and therefore maintain the balance between the excitatory and inhibitory responses necessary to achieve a correlated response to the high intensities at which the courtship song is heard. The preparation in Fig. 3.16.1 shows this "over-inhibition" at a relatively low intensity. In preparations where the inhibition is not so great, this over inhibition is not always obvious because it occurs at intensities beyond those tested and therefore beyond biologically relevent intensities. Despite its strong inhibition to a simulation containing 4.5kHz alone, the preparation in Fig. 3.16.1 is still able to produce a correlated response to the courtship song if the simulation also contains a high frequency component which represents the harmonics of the song. It has been demonstrated in behavioural experiments that a simulation of the calling song containing the harmonics is a more effective attractant than one containing only the carrier frequency (Latimer and Lewis 1986). At the neural level the excitation produced in response to the high frequency component of the courtship song reduces the effect of the high intensity inhibition allowing coding (Fig. 3.16.2). Therefore, there are three components of the courtship song which interact to allow ANA to accurately code its temporal pattern: syllable rate, intensity and frequency.

Outside the strict parameters of the courtship song ANA is capable of responding to a range of stimuli; the

threshold curve shows that it responds to frequencies up to 100kHz and the work of Nolen and Hoy (1984) suggests a role in avoidance behaviour. This neurone cannot therefore be regarded strictly as a temporal filter for the courtship song but within the defined parameters of the songs, its properties make it ideally suited to respond to the courtship song in preference to the calling and aggression songs.

Although no behavioural experiments have been carried out to determine which parameters of the courtship song are important for recogition by the female, much of the work carried out on calling song recognition suggests that syllable rate is an important parameter. Recognition, in behavioural terms, is based on the females' ability to show positive phonotaxis to the sound by either walking or initiating flight towards it. In walking experiments the cricket, usually a female, is placed either on a locomotion compensator i.e. a Kramer treadmill (Kramer 1976; Weber, Thorsen and Huber 1981) often in the dark to prevent reference to visual cues (Stout and Weber 1981), a Y-maze (Popov and Shuvalov 1977) or in an orientation arena (Zaretsky 1972; Stout, DeHaan and McGhee 1983). In flight experiments the cricket is tethered in a wind stream (Moiseff et al. 1978, Pollack and Hoy 1981, Nolen and Hoy 1986). Within these experimental regimes a variety of calling song characteristics have been tested and most of the data indicates that the temporal pattern of the song,

particularly the syllable rate, plays a significant role in the female's ability to recognise its conspecific calling song (Zaretsky 1972; Walker 1957; Ulargaraj and Walker 1975). In fact, experiments carried out on the Gryllus species, suggest that the syllable rate is the only important parameter. A simulation of the calling song presented at the correct carrier frequency with a syllable rate of 30Hz is sufficent to produce positive phonotaxis in the female even if the syllable duration or the duration of the entire song phrase (duty cycle) is altered beyond the natural range (Thorsen et al. 1982; Weber 1984). However, some of these results have now been shown to be somewhat misleading. Most of the above experiments involved a simple choice or no choice paradigm in which temporal parameters were tested individually while other variables where kept constant. Experiments have suggested that a females interpretation of a song pattern may depend on the cricket assessing the "attractiveness" of a variety of parameters of the song (Stout et al. 1983). Doherty (1985a, 1985b) showed that the relative attractiveness of one parameter could be changed by varying the attractiveness of another. He refered to this as the "trade-off" phenomenon. All these experiments involved investigations into behavioural recognition of the calling song. As the physiological results presented here show, it may be reasonable to extend some of these findings to the neural basis of courtship song recognition. Accurate coding of the

trill phrase by ANA is determined by syllable rate, song intensity and frequency: a reduction in intensity can produce coding at a previously unproductive syllable rate (Fig 3.16.1) and an increase in syllable rate can result in coding at an intensity that previously produced a poorly correlated response (Fig 3.16); further, the presence of 16kHz to represent the harmonics of the natural song can produce good coding where the carrier frequency alone did not (3.16.2). To confirm the importance of these parameters in recognition, behavioural experiments must be carried out using simulations of the courtship song to determine their effectivness in phonotaxis. This would fill a gap that is present in the behavioural knowledge because despite the obvious importance of courtship behaviour very little work has been carried out to investigate the major parameters for courtship song recognition.

The importance of intensity in calling song recognition in T. oceanicus has been demonstrated by the behavioural experiments of Doolan and Pollack (1985). They found that at low intensities the females would track song simulations containing a range of temporal patterns but at high intensities, their responses became more selective with the females only performing phonotaxis to songs in which the syllable duration and rate were close to the natural range. Their explanation for this, in behavioural terms, is that at long range, i.e. low intensities, the females may be unable to identify the songs of an

individual male within the background of a congregation of male singing; therefore, the initial attraction may be based on a simple component of the whole sound such as carrier frequency. However at the high intensities heard at close range it is suggested that the females are able to perceive the more complex parameters and recognise individual singing males. This implies that an accurate code of the calling song pattern at high intensities is necessary for the female to distinguish the song of an individual male. At lower intensities both ANA and ANC would be able to respond to the carrier frequency of the song (the simulation of the song in this experiment contained only 4.5kHz). However, at high intensities, the inhibitory response would reduce the response of ANA but allow ANC to code the song. If the males switched to the courtship song at this intensity the rapid syllable rate and reduced intensity of the trill phrase would allow ANA to produce a correlated spike response which ANC would be unable to accurately reproduce. This therefore implies the presence of two pathways: one involved in coding for the calling song in which the predominant neurone would be ANC and one involved in coding the courtship song via ANA.

4.4. Song recognition Based on Two Neuronal Pathways.

A similar two pathway system has been suggested as the mechanism by which Gryllus bimaculatus distinguishes between the courtship and calling songs (Boyan 1981). In

this species AN2, (which is thought to be homologous to ANA), shows some inhibition to low frequency tones and has a best frequency around 15kHz. It has been suggested therefore, that it has a role in courtship song recognition (Wolers and Huber 1978) since the courtship song in this species has a carrier frequency of 14-16kHz. Boyan (1981) showed that this two tone inhibitory effect occured in brain neurones (PABN2) and suggested that interpretation of the songs was based on two neural network systems involving the brain neurones. The function of one system would be to code the high frequency courtship song, mediated by neurones with best frequencies around 15kHz and the function of the other would be to code the low frequency calling and aggression songs, mediated *via* low frequency neurones such as AN1 (Wohlers and Huber 1982) and AN3 (Boyd *et al*. 1984). The high frequency pathway would be most active in the presence of the courtship song and suppressed in the presence of the low frequency calling and aggression songs. The switch between the two pathways is therefore based on frequency and is the result of the two-tone inhibitory effect. The results presented in this project suggest that a similar mechanism occurs in *T. oceanicus* but that in this species the switch is based on the temporal differences between the two songs. ANA is active in the presence of the courtship song where the syllable rate, intensity and frequency content allow accurate coding. In the presence of the calling song however the inhibition

would tend to dominate (because of the higher intensity and slower syllable rate) resulting in a poorly correlated response. ANC however would produce an accurately correlated response to the calling song. Therefore, although the parameters in the two species are different; frequency differences in G. campestris and temporal pattern differences in T. oceanicus; song type recognition appears to be the result of adaptations of the same mechanism.

Both ANA in T. oceanicus and AN2 in G. bimaculatus (Wohlers and Huber 1982; Popov and Markovich 1982) show some variation in their response to low frequency tones; over a range of preparations both neurones show some degree of excitation to 5kHz although the magnitude of the inhibition in ANA in response to high intensities of this frequency, is greater overall than it is in AN2. Therefore, although the switch mechanism, and recognition of the song types, appears to be initiated at the pro-thoracic ganglion level it is likely to be defined more clearly at the higher level brain neurones.

Although this hypothesis suggests a mechanism by which the calling and courtship songs could be distinguished, it gives no indication as to how the crickets recognise their aggression song. In both the Gryllus and Teleogryllus species this song has similar frequency and temporal characteristics to the conspecific calling song. Phonotaxis experiments show that female crickets are attracted by the aggression song. It has been

suggested that, in addition to its role in male-male interactions, it also serves as an indication to the female of the presence of a victorious male (Weber et al. 1981). Final recognition of the song by both males and females may depend on interpretation of its temporal and frequency components and on additional information from antennal and visual contact (Loher and Rence 1978).

The existance of a two pathway system has been used to explain the positive and negative phonotaxis shown by T. oceanicus. Pollack et al. (1984) demonstrated that a female cricket (T. oceanicus), presented with a model of the calling song at 5kHz, showed positive phonotaxis; 5kHz presented in a non-calling pattern (2 pulses/s) resulted in no phonotaxis. If presented at high frequencies (33kHz), both the calling and non-calling song patterns produced negative phonotaxis. At intermediate frequencies (15kHz) the responses of the female were shown to be dependant on the temporal patterns of the stimulus. At 15kHz a model of the calling song produced predominantly positive phonotaxis whereas a non-calling song pattern resulted in negative phonotaxis. In neural terms the low frequency stimulus would stimulate the low frequency pathway, resulting in a coded response by ANC which would be "recognised" in the brain leading to positive phonotaxis in response to the calling song pattern. At 15kHz both ANC and ANA would respond, leading to positive phonotaxis if the stimulus was recognised as the calling song by ANC and negative

phonotaxis if it was interpreted as a predator sound by ANA. This explanation suggests therefore, that ANA has two roles; courtship song recognition and predator detection. Despite the obvious differences in behavioural stratagies necessary for these two functions it is possible for ANA to be involved in both. Negative phonotaxis, considered to represent predator avoidance, is primarily a flight escape behaviour; in walking the movement is often brief and weak (Pollack et al. 1984). Nolen and Hoy (1984,1986b) showed that to initiate negative phonotaxis the activity in Int-1 (likely to be the same neurone as ANA) has to exceed 220 spikes per second. In the context of courtship song recognition the strong inhibition induced by the carrier frequency, greatly reduces the activity in ANA: one spike per syllable results in a rate of around 35 spikes per second. Therefore the switch between the different behavioural stratagies may be based on different rates of activity in ANA.

A general conclusion that can be made from the discussion is that the role of the ascending neurones is to produce a response which represents a template of all, or part, of the stimulus pattern of the incoming sound and then direct this response to a higher level of processing where the signal can be further analysed.

Early research suggested that the response pattern in the ascending neurones of T. oceanicus was matched with an internal template of the conspecific song (Hoy 1978; Hoy

and Paul 1973). However, behavioural tests using randomised simulations suggested this was not the case. The calling song pattern contains three different intervals; the intra chirp, intra trill and inter trill intervals (in the calling song the syllable doublets represent the trill phrase). If the order of presentation of these intervals is randomised while maintaining their correct durations crickets still show positive phonotaxis (Pollack and Hoy 1979). Since these randomised simulations would not correspond to a template of the song it is unlikely that this is the mechanism for recognition.

Recent studies have suggested that there is a signal analyser located within the supra-oesophageal ganglion (brain) of the cricket (Schildberger 1984). Although no recordings have been made from neurones in the supra-oesophageal ganglion in this project, the Lucifer Yellow stain of ANA over its entire course (Fig. 3.6) revealed its anatomy within this ganglion and showed that it arborises extensively within areas similar to those of AN1 and AN2 (Schildberger 1984) and PALF1 and PALF2 (Boyan and Williams 1982) in G. bimaculatus. Although it is difficult to be certain when the neurones are stained in different preparations, the brain neurones, PABN2, in G. bimaculatus (Boyan 1980) appear to have dendritic arborisations in the same areas as the projections from ANA. Two-tone experiments showed that PABN2 neurones are inhibited by low frequencies. This inhibition was assumed

to be pre-synaptic because no IPSP's were present (Boyan 1981). The data presented here shows that the integration between inhibitory and excitatory inputs is performed at the level of the pro-thoracic ganglion. PABN2 neurones are morphologically similar to group "c" of the BNC1 neurones identified by Schildberger (1984). These neurones have dendritic areas which overlap with the dendritic projections of the ascending neurones, AN1 and AN2. Other dendritic areas of these BNC1 neurones overlap with a second group of brain neurones the BNC2 neurones. It is these neurones which appear to analyse the temporal characteristics of the information from the ascending neurones, via the BNC1 neurones (Schildberger 1984). Rather than producing a template of the temporal pattern of the stimuli as the ascending neurones do, the BNC2 neurones respond selectively by producing an increased response to the syllable pattern which most closely represents the natural calling song. Patterns that do not correspond with the conspecific song result in a reduced response. The magnitude of the response is independant of intensity because this parameter has been "stabilised" by the ascending neurones. Schildberger (1985) also showed brain neurones which responded to syllable intervals shorter than those of the calling song but not to longer syllable intervals (a high-pass filter) and other neurones which responded to long intervals but not short ones (a low-pass filter). It has been shown that toads also have neurones

which are "matched temporal filters" to the modulation rate of a white noise stimulus that is close to rate of amplitude modulation in the natural song. In addition there are AM low-pass and high-pass neurones (Rose and Capranica 1984).

Neurones which produce a maximal response to the simulations of the natural song have also been found in the locust brain (Romer and Seikowski 1985). In locusts the duration of the inter-chirp interval has been shown to be the key parameter for song recognition (von Helversen 1972). Using simulations of this song, in which the inter-chirp interval was varied but the chirp structure and spectral composition of the song were kept at the natural level, Romer and Seikowski (1985) recorded from neurones in the brain which responded primarily to stimuli in which the inter-chirp interval corresponded to the conspecific song. The mechanism of temporal selectivity of these neurones was explained as the result of summation of post-synaptic potentials produced by two auditory inputs, one direct input from the ascending neurones and one indirect input via neurones located in the sub-oesophageal ganglion (Boyan and Altman 1985). Because of the different routes the two inputs would be separated temporally and it was suggested that only the conspecific inter-chirp interval would be sufficient to produce summation. With longer intervals the post synaptic potentials would not coincide and with shorter intervals the ascending neurones would show such

strong habituation to the rapidly repeated pulses (Kalmring 1975) that the inputs would not be effective. It is unlikely that this is the mechanism responsible for the temporal filtering in the brain neurones of the cricket. This mechanism in locusts would invariably produce some reproduction of the pattern of the song and there is no indication that the BNC2 neurones in the cricket follow the temporal pattern of the song in any way. In crickets the total number of spikes produced is maximal in response to the correct temporal pattern. Also, to date, no auditory neurones have been found in the cricket which have their cell bodies in the suboesophageal ganglion and, although AN2 (Schildberger 1984), ANA (Fig. 3.6) and Int-1 (Moiseff and Hoy 1983) show some arborisation in this ganglion it is very limited in comparison with their dendritic areas in the pro-thoracic and supra-oesophageal ganglia. Therefore, the mechanisms by which the BNC2 neurones in G. bimaculatus are able to respond specifically to the temporal pattern of the calling song, or by which the brain neurones in T. oceanicus would be able to respond to each of the conspecific songs, are not yet known.

In the cricket the projections of the ascending neurones in the supra-oesophageal ganglion and the processes of the brain neurones are unilateral (Boyan 1980, Schildberger 1984). Observations on T. oceanicus show that males of this species sing in aggregations in close proximity to each other (Cade 1981) therefore the female

must be able to recognise an individual song whithin a group of singing males. In phonotactic experiments females can recognise the conspecific pattern even if a non-specific pattern is presented simultaneously to the other ear (choice paradigm) in a free field situation such that the syllables overlap in time resulting in an overall incorrect temporal pattern (Doherty 1985). Pollack (1986) suggests that this is possible because the ascending neurones and the brain neurones exist as mirror image pairs resulting in two recognition pathways each responding primarly to sound from their ipsilateral side. Comparison between them at a higher level would form the basis of preferential phonotaxis to the conspecific pattern. In contrast, the acridid grasshopper Chorthippus biguttulus does not show this direction specific pattern recognition (von Helversen). Females are unable to show positive phonotaxis to a previously attractive song pattern (no choice paradigm) if the pattern is presented to each ear, out of phase, so that the syllables overlap and obscure the original pattern (choice paradigm). Similarly, two previously ineffective patterns can become effective if, in combination, the result is similar to the natural pattern. There is therefore, some addition of input before pattern recognition takes place in the CNS (von Helversen 1984). However, in the natural situation this addition of inputs may not arise. Although, like crickets, male grasshoppers tend to sing together, adjacent males will delay their

singing with respect to their immediate neighbours, with the result that there are obvious periods in which only one male is singing. This tendency to delay singing does not occur in crickets although the males in some species avoid obstruction of the song patterns by singing in synchronisation (Walker 1969, Heiligenberg 1969). Bushcrickets also synchronise their songs (Samways 1977; Latimer 1981). These observations suggest that behavioural differences form the basis of a different mechanism by which the brain neurones in the cricket (Schildberger 1985) and the locust (Romer and Seikowski 1985) tune their response to the temporal pattern of the song.

Although the above disscussion has sought to explain phonotaxis on the basis of song recognition involving the ascending neurones it is unlikely that they are the only neurones in the pro-thoracic ganglion that are involved. One of the obvious other candidates are the omega neurones (Fig. 3.17).

4.5. Physiology of ON1.

The threshold curve (Fig. 3.18) shows that best frequency of ON1 is at the carrier frequency of the songs. When presented with simulations of the natural songs ON1 produced an accurately correlated response to the syllable pattern of all three songs, particularly the calling and aggression songs.

The first recording (extracellular) of this cell in

T. oceanicus was by Casaday and Hoy (1977). It was refered to as Int-2 since it was the second neurone identified in this species at the time. However, anatomically and physiologically it is similar to ON1 and is therefore almost certainly the same cell. An homologous neurone in G. bimaculatus was originally described as a Local Segmental Auditory Neurone (LSAN: Popov, Markovich and Andjan 1978) but in both G. bimaculatus and G. campestris, Wohlers and Huber (1978) refered to it as the omega neurone because of its shape. This nomenclature was changed to ON1 when a second omega neurone type was discovered (Wohlers and Huber 1982) and this is the terminology adopted throughout this thesis.

In some respects ON1 is physiologically similar to ANC. Both are tuned to 5kHz. However ANC is a low frequency neurone whereas ON1 shows an excitatory response up to 100kHz (Fig. 3.18). Popov et al. showed that LSAN was also tuned to 5kHz and produced a response up to at least 40kHz. However, the threshold curve plotted for the omega neurone by Wohlers and Huber (1978) showed that although its best frequency was at 4.5kHz it only responded up to 16kHz.

As described in section 2, all the neurophysiological experiments in this project were carried out under free field conditions with the result that although the sound stimulus was presented at 90° to the one side of the cricket's body, sound had access to both ears.

Extensive studies have been carried out on ON1 in

the Gryllid species. Under free field conditions ON1 in both G. campestris and G. bimaculatus shows a reduction in sensitivity of 10-12dB when sound is presented contralateral to its cell body side compared to its response to ipsilateral stimulation (Wohlers and Huber 1978). When investigated under closed field conditions, in which the ears were acoustically isolated by severing the acoustic trachea which couples them and each ear was stimulated individually using leg phones (Kleindienst et al. 1981), intracellular recordings from ON1 in G. campestris and G. bimaculatus showed that the excitation produced in response to ipsilateral stimulation was replaced by inhibitory post synaptic potentials when the sound was presented exclusively from the contralateral side (Wohlers and Huber 1982). This led to the sugestion that the mirror image ON1 was mediating an inhibitory effect on its partner and that these neurones were therefore capable of showing mutual inhibition (Wohlers and Huber 1982, Kleindienst et al. 1981). This was confirmed by Selverston et al. (1985) who showed that the inhibition, recorded from the omega neurone contralateral to the sound source, was abolished if the ipsilateral ON1 was killed. The inhibition was in fact replaced by a weak excitatory response. Selverston et al. suggested that the origin of this excitation was the result of either imperfect acoustic isolation between the two ears, primary afferent neurones crossing the midline (which has never been observed: Esch

et al. 1980; Eibl and Huber 1979) or another intermediate interneurone. However, the most likely explanation is that it is the result of excitatory input, from the primary afferents ipsilateral to the sound source, onto the dendritic area of the intact omega neurone contralateral to its cell body. It is generally assumed that the two dendritic areas of ON1 are functionally distinct: the cell body side area represents the input side whereas the contralateral area represents the output side. Recordings from the dendritic area ipsilateral to the cell body show spikes imposed on EPSPs (Fig. 3.19(i)) and, in general, those from the contralateral dendritic area show only spikes since the EPSP does not travel the length of the axon (Fig. 3.19(ii)). However, in a few preparations some EPSP activity is present in the contralateral area (Fig. 3.19(iii)) indicating that there is some input from the primary afferents on this side of the ganglion. Anatomical results also support this sugestion. Fig. 3.20 shows a stained omega neurone and a primary afferent neurone associated with its contralateral side. This has also been shown in G. campestris (Wohlers and Huber 1985). In four other preparations primary neurones were also filled in association with the recorded omega neurone (Fig. 3.20.1). In two of these preparations the primary neurone was associated with the contralateral dendritic field. In one of these preparations the recording was made from the dendritic field of ON1 ipsilateral its cell body so the

electrode could not have been responsible for staining the primary neurone. The dye must therefore have spread from the injected cell directly to the primary neurone. Dye-coupling is common (Glautz and Kirk 1981), although not exclusive to, cells which have electrical synapses (Stewart 1978; 1981) so this suggests the possibility of gap junctions between these two cells with the primary neurone mediating an excitatory input to the "output" area of ON1.

The ON1 ipsilateral to the sound source often showed a reduction in response to a low frequency tone when it was presented at a very high intensity compared to its response to lower intensities. This decrease in response was abolished when the preparation was bathed with Picrotoxin (Fig. 3.21). It was therefore the result of a neurally mediated inhibitory input.

4.5.1. Role of ON1 in song recognition.

When presented with simulations of the natural songs ON1 produces an accurately synchronized spike response to the syllable patterns of the songs, in particular the calling and aggression songs (Fig. 3.22). The homologous neurone in G. bimaculatus and G. campestris accurately codes the syllable patterns of the conspecific calling songs (Popov et al. 1978, Wohlers and Huber 1982). This suggests the ON1 neurones are involved in the recognition process of these songs. Wiese and Eilts (1985), in experiments on G. bimaculatus, have taken this a stage

further. They suggest that the mutual inhibition shown by the paired ON1 cells acts as a temporal filter specifically tuned to the syllable pattern of the calling song. Isolated stimulation with a 4.5kHz tone *via* a leg phone produced a burst of spikes in the omega neurone with cell body ipsilateral to the stimulated ear. This excitation produced corresponding inhibition in the contralateral omega neurone after a 1ms delay. This delay represents conduction time and synaptic delay. The inhibitory response consists of a hyperpolarisation followed, approximatly 15ms later, by a post inhibitory rebound depolarisation (PIRD). Wiese and Eilts-Grimm (1985) suggested that this depolarisation initiates a feedback inhibitory loop from the contralateral omega neurone back to the ipsilateral omega neurone mediated *via* the PIRD. This depolarising potential then induces an inhibitory effect on the ipsilateral omega neurone. It is suggested that this inhibition has a duration of 15ms during which time the ipsilateral ON1 is unable to respond to further stimulation. Therefore this 15ms delay occurs twice in the system; once during the initial inhibition mediated on the contralateral ON1 and once on the ipsilateral ON1 mediated *via* the PIRD. Wiese suggests that this total delay of 30ms corresponds to the inter-syllable duration of the conspecific calling song and therefore that this circuit provides the basis for a preferential response to the conspecific song. However this mechanism assumes that the PIRD is sufficient to produce a

feedback inhibitory effect on the ipsilateral omega neurone which is of similar magnitude to the initial inhibitory effect on the contralateral omega that was mediated by a burst of action potentials in the ipsilateral ON1. It also assumes that subthreshold activity is transmitted across omega neurones from one dendritic field to another. Although Wohlers and Huber (1978) showed the presence of some subthreshold activity in recordings from the dendritic area contralateral to the cell body its amplitude was reduced to about 1/5 of that of the ipsilateral EPSP. In the majority of recordings from this area in T. oceanicus only action potentials were seen (Fig. 3.19(ii)). If this type of temporal filtering mechanism exists it would only be appropriate for the calling song of G. campestris and G. bimaculatus. The pattern of the calling song in T. oceanicus or in A. domesticus does not fit with the inherent temporal characteristics of this mechanism.

It is generally accepted, because of their morphology and mutual inhibitory mechanisms, that the ON1 neurones are involved in sound localisation (Casaday and Hoy 1977, Wohlers and Huber 1982, Popov et al. 1978, Kleindienst et al. 1981). Crickets are unable to orientate to sounds presented anteriorly. To obtain directional information they approach a sound source in a zig-zag fashion in order to bring it at least 15 degrees lateral to the long axis of the body. (Latimer and Lewis 1986).

With sound presented off centre, the omega neurone nearest the sound source recieves greater input than its partner. In this free field situation, the most strongly stimulated ON1 has an inhibitory effect on the other omega, increasing the binaural contrast between the directional cues recieved at each ear (Wohlers and Huber 1978). Localisation accuracy is highly frequency dependant (Hill 1974, Boyd and Lewis 1984). The calling songs of T. oceanicus, A. domesticus and both G. bimaculatus and G. campestris have carrier frequencies around 4.5kHz. This frequency has a relatively large wavelength compared to the size of cricket. Therefore it can obtain very little directional information from the diffraction of sound around the body. However, behavioural experiments show that crickets produce the most accurate orientation to songs containing the correct carrier frequency (Oldfield 1980) and recordings from the auditory nerve show that the maximum left/right differences are obtained in response to frequencies close to the carrier frequency (Boyd and Lewis 1984). Crickets achieve the inter-aural intensity differences necessary to allow localisation because of the specific properties of their auditory apparatus. The tympanal membranes on the tibiae of each fore-leg are acoustically coupled via a tracheal network (Hill and Boyan 1976,1977) which also opens at two auditory spiracles situated on the prothorax. There are therefore four potential access points for sound entry (Larsen and Michelsen 1978). Each ear acts as a pressure

difference reciever where the net response depends on the sound impinging directly onto the outer surface of the posterior tympanal membrane and the sound conducted through the acoustic trachea from the contralateral posterior tympanal membrane and the two acoustic spiracles (Larsen 1981). Blocking experiments show that the spiracles, in particular the ipsilateral spiracle, are more important than the contralateral posterior tympanal membrane as an entry point for sound (Boyd and Lewis 1984). Enhancement of these directional cues by mutual inhibition of the omega neurones may provide the insect with information to allow more accurate localisation of the sound source.

However, recent behavioural experiments throw some doubt on this suggestion. A. domesticus, in which one of the omega neurones has been killed, produce errors in phonotaxis consistant with a lack of inhibitory input to one side; but this is only shown when the syllable rate of the song simulation is varied. If the simulation is presented at the natural syllable rate the cricket shows accurate orienation. If both neurones are killed phonotaxis is not affected at all (Atkins, Ligman, Burghardt and Stout 1984).

Most of the work on the mutual inhibitory effect of omega neurones has been carried out on the Gryllus species. It is possible therefore that the situation is different in A. domesticus. Further investigations are necessary but because the specific function of most of the

auditory neurones, and the connections between them, are not yet sufficiently well known, behavioural experiments in which one cell is killed may produce misleading results.

The role of the inhibition of ANA in courtship song coding has been described. Since the omega neurones have an inhibitory effect on each other it seemed plausable that the neurally mediated inhibitory effect seen in the response of ANA to low frequency sounds was mediated *via* the omega neurones. Throughout these experiment the terminology adopted refers to the position of the omega neurone in relation to the dendritic area of ANA: the ipsilateral ON1 has its input dendritic area (cell body side) ipsilateral to the dendritic area of ANA (Fig. 3.31(i)): the contralateral ON1 has its input dendritic area contralateral to the dendritic area of ANA (Fig. 3.31(ii).

Investigating the synaptic connectivity between the omega neurones and ANA involved manipulating the response of the omega neurone with current injection while monitoring any following change in the responses of ANA through a suction electrode. The presence of the response of ANA in the suction electrode was confirmed by recording from the suction electode while simultaneously recording the response of ANA *via* an intracellular electrode (Fig. 3.30(i)). The corresponding spike responses from the electrodes showed that the same unit was being recorded in

both (Fig 3.30(ii)) and subsequent staining of the intracellular unit revealed that ANA was the neurone being recorded. The reduction of the response of ANA in the presence of hyperpolarising current shows that current manipulation does affect the response of the neurone (Fig 3.30(iii)).

4.6. Investigations into synaptic connectivity between the ipsilateral ON1 and ANA.

Anatomical and physiological evidence from early experiments suggested that the ipsilateral omega neurone was mediating the inhibition. A cobalt chloride stain of ANA together with the output dendritic area of the ipsilateral ON1 (Fig 3.32) suggested the presence of a connection between the distal dendritic fibres of both neurones across the midline of the ganglion. Experiments in which the input to the leg contralateral to the dendritic area of ANA was abolished, by either cutting the leg nerve close to the ganglion or by amputating the leg at the coxa, showed no effect on the inhibitory response of ANA to low frequencies (Fig 3.33). This suggests that the inhibition is ipsilateral in origin. Similar results were reported previously by Hutchings and Lewis (1984) on this species. Moiseff and Hoy (1983) also showed that the presence of the ipsilateral ear was necessary for the inhibition in Int-1 (T. oceanicus). Conversly, in G. bimaculatus Popov and Markovich (1982) showed that the

inhibition of HF1AN was mediated primarily *via* the contralateral ear. This therefore represents a major difference in the physiology of the high frequency ascending neurones in these two species.

Simultaneous recordings from both ANA and the ipsilateral ON1 in the same preparation were used to investigate the presence of inhibitory synaptic coupling between these neurones. Comparison of the responses of these neurones in early results suggests the presence of a connection: ON1 shows a strong excitatory response to low frequency tones that produce an inhibitory response in ANA, whereas high frequencies that produced excitation in ANA result in a much reduced response in ON1 (Fig 3.34).

However, later experiments revealed inconsistancies between the responses of the neurones. As mentioned previously the threshold curve of ON1 in *T.oceanicus* shows that this neurone responds to frequencies up to 100kHz (Fig 3.18) and its sensitivity to frequencies above 8kHz is very similar to that of ANA (Fig 3.35). This is unlike the situation in *G. campestris* where ON1 is essentially a low frequency neurone that does not respond to frequencies above 16kHz (Wohlers and Huber 1978). In *T. oceanicus* therefore the magnitude of the response of ON1 and ANA to a high frequency tone is very similar, a finding which does not support the suggestion that ON1 mediates the inhibition on ANA.

Another inconsistancy was revealed when the

responses of ON1 and ANA to a high frequency tone in the presence of a range of intensities of a low frequency tone were compared. Initially the response of ON1 increases as the intensity of the tone is increased. However at very high intensities, when the response of ANA is increasingly inhibited the response of ON1 is also reduced (Fig 3.36) Similarly ON1 shows a decrease in response to very high intensities of a low frequency tone (Fig 3.36.1).

Current manipulation experiments were carried out on these two neurones. The ipsilateral ON1 was assumed to be the presynaptic cell and was recorded with an intracellular electrode to allow manipulation of its response by the injection of current. An extracellular suction electrode, placed over the cut end of the cervical connective, monitored the responses of ANA throughout the enforced changes in the response of the ipsilateral ON1. Injection of either hyperpolarising (Fig 3.37) or depolarising current (Fig 3.38) greatly affects the response of ON1 to high frequency tones presented alone, or together with a low frequency tone. There is however no corresponding alteration in the response of ANA.

The same technique, used in locusts (Marquart 1985) revealed a clear inhibitory connection between two interneurones: a local auditory interneurone (SN1) and an ascending neurone (AN1). Injection of depolarising current into SN1 in the presence of a high frequency tone, which in the intact preparation produced a burst of spikes in AN1

but no response in SN1, artificially induced spiking in SN1 which in turn resulted in complete inhibition of the response of AN1 to the tone. Therefore, if there was a connection between the contralateral ON1 and ANA, this type of experiment should have revealed it.

4.7. Investigations into synaptic connectivity between the contralateral ON1 and ANA.

The possibility remained that the contralateral ON1 was the source of the inhibitory effect on ANA. Anatomically this appeared more likely, since the output field of the ON1 lies adjacent to the input area of ANA (Fig 3.31(ii)). In addition recent experiments carried out on G. campestris (Selverston et al. 1985) have shown the presence of an inhibitory connection between the ipsilateral omega neurone and AN2, the supposed homologue of ANA.

However current manipulation experiments in T. oceanicus showed that despite suppressing the response of the contralateral ON1 with hyperpolarising current ANA continued to show an inhibitory response to low frequency tones (Fig 3.39). Similarly an increase in ON1's response by injection of depolarising current failed to initiate an inhibitory response in ANA (Fig 3.40).

Thus the results of current manipulation experiments on both the ipsilateral and contralateral ON1 neurones, in T. oceanicus, show that neither of these neurones is

responsible for mediating the inhibition on ANA. There is, therefore, a conflict of results between the two species G. campestris and T. oceanicus. This conflict is unlikely to be the result of differences in experimental techniques. Comparison of the physiological properties of the neurones involved suggest that there is a fundamental difference in the processing of auditory information between these two species.

The experiments of Selverston et al. (1985) involved making single cell ablations using a photoinactivation technique developed by Miller and Selverston (1979). This technique showed that single cells filled intracellularly with a photoabsorbtive dye (Lucifer Yellow CH) could be killed by irradiating the ganglion with high intensity blue light. Lucifer Yellow (CH) has peak absoption at 426nm (blue: Stewart 1978). During the dye-killing experiments, the contralateral omega cell was stained with Lucifer Yellow. The responses of ANA were recorded in the intact preparation and after the stained ON1 was irradiated and killed. Prior to the killing of the contralateral ON1, AN2 showed IPSP's in response to 5kHz presented to the ear contralateral to its dendritic area. After killing the contralateral ON1 this inhibition was removed and replaced by a weak excitatory response. This showed that the inhibition on AN2 was mediated via the contralateral ON1. The excitation, normally masked by the inhibitory response, was explained as the result of input from primary neurones

on the stimulated side to a small dendritic area of AN2 situated at the base of the neurite and contralateral to the main dendritic process. This area is also present in ANA (Fig 3.5).

The experiments carried out in this project, to investigate the presence of an inhibitory connection between ANA and ON1 in T. oceanicus, involved the use of current manipulation experiments rather than dye-killing. The difference between manipulating the response of a neurone compared to killing it by photoinactivation is that when killed, the neurone no longer produces any response: during current manipulation experiments on the other hand, although the spiking response can be completely suppressed, invariably the EPSP activity remains. Selverston et al. (1985) suggested that EPSP activity in one cell is sufficient to mediate a corresponding response in an associated cell. If this is the case it is possible that in the current manipulation experiments the EPSP in ON1 , still present after suppression of the spiking activity, may be capable of mediating an inhibitory effect on ANA. However, it is certain that any inhibition mediated by this EPSP activity would be far less than that mediated by spiking. Therefore, if there was a connection between the two cells the magnitude of the overall response would be vastly reduced if the spiking activity was supressed. No such reduction was seen in the response of ANA to any intensity of low frequency tones.

At first sight, this conflict of results between T. oceanicus and G. bimaculatus appeared surprising. However, comparison of the responses of the "homologous" neurones between the two species reveals differences in their physiological properties which may explain it. The experiments carried out on G. bimaculatus (Selverston et al. 1985) involved sound presented within a closed system in which each ear was acoustically isolated (Kleindienst et al. 1981). Under these conditions, although AN2 shows a high degree of variability in response to low frequency tones, it commonly produces an inhibitory response to sound presented to the ear contralateral to its dendritic field. Sound presented either ipsilaterally or bilaterally produces an excitatory response in AN2 (Wohlers and Huber 1982). This is very different from the physiology of ANA. Presentation of a low frequency tone under free field conditions produces IPSPs in ANA even when the sound is presented predominantly from the side contralateral to its dendritic field. An excitatory response is only produced if the intensity of the low frequency tone is greatly reduced. Therefore, inhibition is produced in ANA to a much greater degree than in AN2.

The physiology of ON1 also differs between the two species. In G. bimaculatus, ON1 is essentially a low frequency neurone with a best frequency at 5kHz but showing no response to frequencies above about 16kHz (Wohlers and Huber 1978). In T. oceanicus ON1 also has a best frequency

at 5kHz but responds to frequencies up to 100kHz. A plot of the threshold curves from ANA and ON1 in the same preparation shows that they have similar thresholds of response to frequencies above 10kHz (Fig 3.35). Therefore, at low frequencies the opposing responses of excitation in ON1 and inhibition in ANA are consistant with the possibility that the omega neurone is mediating the inhibition. However at high frequencies both ON1 and ANA show excitation. Since, in G. bimaculatus, ON1 does not respond to high frequencies its physiology is compatable with that of AN2.

Since ON1 does not mediate the inhibitory effect on ANA it must originate elsewhere. One possibility is that the inhibiton is mediated directy via the primary afferent neurones. In T. oceanicus groups of primary afferents respond to a range of frequencies between 0.5kHz and 42kHz (the highest frequency tested: Hutchings and Lewis 1981). Some are specifically tuned to the 4.5kHz carrier frequency of the songs. In G. campestris, primary neurones tuned to this frequency project towards the centre of the ganglion (Eibl and Huber 1979, Esch et al. 1981) where they arborise within the areas typically occupied by the dendritic field of AN1, and in T. oceanicus, ANC. Two separate groups of afferents tuned to 4.5kHz but with different absolute sensitivities, or different magnitudes of suprathreshold response, could represent the excitatory and inhibitory inputs at this frequency. Latency measurments support the

suggestion of a direct inhibitory input from the primary neurones. Using a sound stimulus, it is difficult to calculate absolute values for the latencies of the response because the nature of the stimulus demands that certain assumptions are made: to prevent a click onset the pulse had a 5ms rise/fall time and to represent the free field situation the loud speaker was positioned approximately 1m from the preparation. To facilitate comparison between preparations the latency of response was calculated as the distance between the onset of the stimulus and the start of the response, minus the 5ms sound propagation time from the speaker to the preparation. Although the values are not accurate, comparison of the responses in the same preparation shows that the latencies of both the excitatory and inhibitory responses in ANA are very similar (approximatly 10ms). The excitatory input to the ascending neurones in G. campestris is considered to be mono-synaptic. Therefore this suggests that the inhibitory response in ANA is also mono-synaptic. However, the latency measurements may not be not accurate enough to be certain of this. The inhibitory response of an ascending neurone in the locust (AN1), shown to be mediated via a local interneurone (SN1: Marquart 1985) has a latency of 5ms more than the excitation (Romer and Marquart 1984). In bushcrickets the inhibition shown by the ascending neurones can be delayed by 20ms relative to the excitatory response (Oldfield and Hill 1983). These latencies suggest

polysynaptic connections. The inhibition on cricket ascending neurones may also be mediated *via* an intermediate neurone but have a much shorter latency. Weise (1985) for example showed that the latency of the inhibitory response of one ON1 neurone (produced by the activity of its mirror-image partner) was delayed by only 1ms relative to the latency of the excitatory response of the partner. Therefore, latency measurments of this type make it difficult to determine the presence or absence of an intermediate neurone. Intracellular experiments involving current manipulation of the response of a single primary neurone while monitoring the response of ANA may provide further information. However, since a group of primary neurones is likely to be involved, manipulation of the response of one may not be sufficient to reveal a corresponding effect on ANA.

4.8. The physiology of ON2.

If an intermediate neurone is responsible for mediating the inhibition, the only other identified local neurone in *T. oceanicus* is the second type of omega neurone, ON2. Anatomically, the main difference between the two omega neurones is that ON2 has a large dendritic branch which projects across the centre of the ganglion from the dendritic area ipsilateral to the cellbody to the contralateral dendritic area. This branch is not present in ON1. In addition, the diameter of the

axon in ON2 is greatly reduced compared to ON1 (Fig 3.23). These features agree with the anatomical details described for ON2 in G. campestris (Wohlers and Huber 1982). ON2 has also been identified in Acheta domesticus (Atkins et al. 1985). Although this is the first description of ON2 in this species no anatomical or physiological detail are given therefore it is presumed that the properties of this neurone in A. domesticus are the same as those published for ON2 in G. campestris.

Physiologically, ON1 and ON2 differ greatly. The results recorded from T. oceanicus show that in this species ON2 is non-spiking. In response to a range of frequencies it produces a compound EPSP which reaches amplitudes of around 25mV in some cases. This depolorisation is sufficient to cross spike threshold in ON1, but in ON2, no spikes were ever produced (Fig. 3.25). In contrast, in G. bimaculatus Wohlers and Huber (1982) showed that ON2 responded to both ipsilateral and contralateral stimulation with an average of 2/3 spikes per sound pulse. When the sound pulses were arranged in a simulation of the conspecific calling song the ON2 response did not accurately follow the syllable pattern. In T. oceanicus the EPSP's did show a good correlation in response to the syllable structure of the songs (Fig 3.26). Atkins et al (1985) suggested that ON2 in A. domesticus played a role in the song coding of this species. The significant differences between the properties of ON2 in

different species suggest they may have different functions. Non-spiking interneurones are well documented in the motor pathways of insects (Burrows 1979.1981: Pearson and Fourtner 1974) and neurones which transmit information via graded potentials are known in the visual system of locusts (Simmons 1982) but this is the first description of a non-spiking neurone in the insect auditory system. Although the graded transmitter release typical of these neurones suggests ON2 may have a modulatory effect on other auditory neurones, a great deal more information is needed before ON2 can be ascribed a function. It is also difficult at this time to give an explaination as to why it is non-spiking in T. oceanicus while showing a spiking response in other species.

4.9. Partially identified neurones.

In addition to the auditory neurones already described a few preparations revealed information on two other groups of auditory neurones which are likely to have some role in the processing of conspecific information. These are refered to as the through neurones and the descending neurones.

4.9.1. Characteristics of Through Neurones.

In one preparation an extracellular recording from the cervical connectives revealed the anatomical and physiological properties of a through neurone (Fig 3.27).

Morphologically it is similar to TNB, one of five groups of through neurones previously identified in T. oceanicus (Hutchings: unpublished data). It has both an ascending and a descending axon from which bilateral dendritic areas project. Although some of these dendritic areas are located within the auditory neuropiles the majority extend to other areas. This suggests that this neurone receives more than one input and is probably bi- or multi-modal. Only auditory stimuli were presented to this neurone and it showed an excitatory response to frequencies between 8kHz and 90kHz and some degree of two-tone suppression in response to low and high frequency stimulation. Two-tone suppression effects were typical of all the through neurones described in T. oceanicus by Hutching and Lewis (1984). In G. campestris an identified through neurone, designated TN1 (Wohlers and Huber 1982), was shown to respond to both auditory and vibratory stimuli (Silver, Kuhne and Lewis 1984; Kuhne et al. 1984). This neurone habituates rapidly to auditory stimuli when presented alone but if an additional vibratory stimulus is introduced this habituation is largely overcome (Kuhne et al. 1984). Similarly, a vibratory input may prevent the inhibition shown in TNB. Bifunctional neurones are common in locusts and bushcrickets (Kalmring and Kuhne 1980; Kalmring et al. 1983; Kalmring 1983). In these insects it is thought that their response to the vibrations produced by the males when they stridulate (Kemper and Kuhne 1983) reinforces the

auditory response to the songs and improves the receivers' ability to localise the sound (Latimer and Shatral 1983). Although in some environments, particularly in dense woody bushes, vibration signals can be propagated up to 2m, in other habitats such as grasslands the vibration signals have relatively short propagation distances (Keuper, Otto, Latimer and Schatral 1985) and therefore may be restricted to short range communication. Because of their responses to vibratory tones, through neurones may be primarily involved in short range courtship behaviour (Kuhne et al. 1984)

4.9.2. Characteristics of Descending Neurones.

Intracellular experiments revealed two types of descending neurones in T. oceanicus. One of these (Fig 3.28) was morphologically similar to an indentified neurone in DN1, G. campestris (Wohlers and Huber 1982). The cell body of the neurone is situated in the anterior quadrant of the ganglion contralateral to this neurone's dendritic arborisations which lie within the lateral and medial parts of the auditory neuropile. This descending neurone in T. oceanicus was not held for long and it was therefore only tested with 4.5kHz. It showed a strong excitatory response to this frequency with a threshold level of approximatly 44dBSPL. Wohlers and Huber (1982) used isolated stimulation of each ear to show that DN1, in G. campestris, also produced an excitatory response to 4.5kHz but only when presented to the ear contralateral to the cell body.

Ipsilateral stimulation produced no response suggesting that the contralateral dendritic field is the input area and that it recieves its input only fron the contralateral primary neurones. This frequency was presented as a simulation of the calling song of G. campestris and the response of DN1 was correlated with the temporal pattern of the syllables in the song. Since the carrier frequency of the calling song in T. oceanicus is also 4.5kHz the descending neurone in this species may also be involved in recognition of this song. Recordings from the connectives between the prothoracic and mesothoracic gangila of T. oceanicus (Pollack 1983) have shown the presence of a group of descending neurones sensitive to frequencies between 20-50kHz. Because of their preferential responses to either ipsilateral or contralateral stimulation, Pollack suggested that they had a role in directional hearing. Since no anatomical detail was given, some of these recordings may have been taken from the desending axons of through neurones rather than true descending neurones.

A descending neurone recorded in the other Australian species, T. commodus (Boyan 1978,1979b) may be homologous to DN1. It is refered to as the D neurone and although once again there is no anatomical detail, physiologically it appears similar to DN1. However, its threshold curve shows that in addition to producing a peak response to 4.5kHz it is also sensitive to approximately 900Hz which suggests that there is a vibratory input. Boyan

suggests that the main function of the D neurone is in directional coding of information and possibly involves direct interactions with the thoracic motor systems without the involvement of supra-oesophageal (brain) neurones. Similar isolated systems exist in Tettigonids (Mckay 1970).

The second type of descending neurone recorded was anatomically different (Fig 3.28). However no physiological information was obtained. An interesting feature of this neurone is that unlike all the other prothoracic auditory neurones, its cell body is situated in a posterior quadrant.

More detailed investigations into the functions of the descending and through neurones may provide useful information on their relationships, either direct or indirect, with motor neurones.

CHAPTER 5.

CONCLUSIONS.

Recognition of the song type in T. oceanicus must be based on differences in the temporal patterns of their three conspecific songs. The neurophysiological investigations carried out in this project have identified neurones which because they are capable of producing an accurately correlated response to the temporal patterns of the songs are likely to be involved in the pathways responsible for their recognition. These neurones, ANC which codes the calling song and ANA which produces a correlated response to the courtship song, form the basis of two neuronal networks which are likely to involve other pro-thoracic neurones as well as higher order neurones in the brain. The evidence presented here suggests that ANA acts, to some extent, as a temporal filter for the courtship song. However, refinement of this filtering and final recognition of the song type may involve the neurones in the supra-oesophageal ganglion. Neurones at this level must also activate the neural pathways responsible for initiating the appropriate behavioural response. Further investigations, particularly into the origin of the inhibition on ANA would result in better understanding of the neural pathways and the mechanisms by which the the conspecific songs are recognised

The aim of this thesis has been to relate the physiology of identified auditory neurones to the known acoustic behaviour of crickets. The possibility of specific roles for these neurones has been advanced however, since

central nervous systems act in an integrated fashion it is unlikely that these are the only neurones involved. Nevertheless, the ability to relate neurophysiological investigations to simple fixed action patterns greatly contributes to the understanding of the neural basis of behaviour.

REFERENCES.

Alexander, R.D. (1961): Aggressivness, territoriality and sexual behaviour in field crickets (Orthoptera, Gryllidae). Behaviour. 17: 130-223.

Atkins, G., Ligman, S., Burghardt, F. and Stout, J.F. (1984): Changes in phonotaxis by the female cricket Acheta domesticus L. after killing identified acoustic interneurones. J. Comp. Physiol. A. 154: 795-804.

Bacon, J.P. and Altman, J.S. (1977): A silver intensification method for cobalt filled neurones in wholemount preparations. Brain Res. 138: 359-363.

Bailey, W.J. (1970): The mechanics of stridulation in bushcrickets (Tettigonioidae, Orthoptera). 1. The tegminal generator. J. Exp. Biol. 52: 495-505.

Bailey, W.J. and Broughton, W.B. (1970): The mechanics of stridulation in bushcrickets (Tettigonioidae, Orthoptera) II. Conditions for resonance in the tegminal generator. J. Exp. Biol. 52: 507-517.

Ball, E.E. and Hill, K.G. (1978): Functional development of the auditory system of the cricket Teleogryllus commodus. J. Comp. Physiol. 127: 131-138.

Belton, P. (1974): Analysis of direction finding in male mosquitoes. In. Experimental Analysis of Insect Behaviour. Ed. L. Barton Browne. Springer Verlag, Berlin, Heidleberg and New York.

Bennet-Clark, H.C. (1970): The mechanisms and efficiency of sound production in mole crickets. J. Exp. Biol. 52: 619-652.

Bennet-Clark, H.C. (1975): Sound production in insects. Sci. Prog. Oxf. 62: 263-283.

Bennet-Clark, H.C. and Ewing, A.W. (1969): Pulse interval as a critical parameter in the courtship song of Drosophila melanogaster. Anim. Behav. 17: 755-759.

Bentley, D.R. and Hoy, R.R. (1972): Genetic control of the neural network generating cricket (Teleogryllus, Gryllus) song patterns. Anim. Behav. 20: 478-492.

Boyan, G.S. (1978): Coding of directional information by a descending interneurone in the auditory system of the cricket. Naturwissenschaften 65: 212-213.

Boyan, G.S. (1979a): Directional responses to sound in the central nervous system of the cricket Teleogryllus commodus (Orthoptera: Gryllidae). I. Ascending interneurones. J. Comp. Physiol. 130: 137-150.

Boyan, G.S. (1979b): Directional responses to sound in the central nervous system of the cricket Teleogryllus commodus (Orthoptera: Gryllidae). II. A descending interneurone. J. Comp. Physiol. 130: 151-159.

Boyan, G.S. (1980): Auditory neurones in the brain of the cricket Gryllus bimaculatus (DeGeer). J. Comp. Physiol. 140: 81-93.

Boyan, G.S. 1981. Two-tone suppression of an identified auditory neurone in the brain of the cricket Gryllus bimaculatus (DeGeer). J. Comp. Physiol. 144: 117-125.

Boyan, G.S. and Altman, J.S. (1985): The suboesophageal ganglion: a "missing link" in the auditory pathway of the locust. J. Comp. Physiol. A. 156: 413-428.

Boyan, G.S. and Williams, J.L.D. (1981): Descending interneurones in the brain of the cricket: Anatomy and responses to auditory stimuli. Naturwissenschafften. 68: 486-487.

Boyan, G.S. and Williams, J.L.D. (1982): Auditory neurones in the brain of the cricket Gryllus bimaculatus (DeGeer): Ascending interneurones. J. Insect Physiol. 28: 493-501.

Boyd, P.J. and Lewis, D.B. (1982): Auditory localisation in the bushcricket Tettigonia cantans (Orthoptera, Tettigoniidae). Acoustic Letters 6: 6-10.

Boyd, P. and Lewis, B. (1983): Peripheral auditory directionality in the cricket (Gryllus campestris L., Teleogryllus oceanicus Le Guillou). J. Comp. Physiol. A. 153: 523-532.

Boyd, P., Kuhne, R., Silver, S. and Lewis, B. (1984): Two-tone suppression and song coding by ascending neurones in the cricket Gryllus campestris L. J. Comp Physiol. A. 154: 423-430.

Broughton, W.B., Samways, M.J. and Lewis, D.B. (1975): Low frequency sounds in non-resonant songs of some bushcrickets (Orthoptera, Tettigonioidae) Ent. et appl. 18: 44-54.

Burrows, M. (1979): Graded synaptic interactions between local premotor interneurones of the locust. J. Neurophysiol. 42(4): 1123-1148.

Burrows, M. (1981): Local interneurones in insects. In. Neurones Without Impulses: Their significance for vertebrate and invertebrate nervous systems. Ed. A. Roberts and B.M.H. Bush. Society for Experimental Biology. Seminar series 6. Cambridge University Press.

Busvine, J.R. (1955): Simple methods for rearing the crickets (Gryllus domesticus) with some observations of speed of development at different temperatures. Proc. R. Ent. Soc. Lond. (A). 30: 15-18.

Cade, W.H. (1981): Field cricket spacing and the phonotaxis of crickets and parasitoid flies to clumped and isolated cricket songs. Z. Tierpsychol. 55: 365-375.

Cade, W.H. and Wyatt, D.R. (1984): Factors affecting calling behaviour in field crickets, Teleogryllus and Gryllus (age, weight, density and parasites). Behav. 88(1-2): 61-67.

Campbell, D.J. and Shipp, E. (1979): Regulation of spatial pattern in populations of the field cricket Teleogryllus commodus (Walker). Z. Tierpsychol. 51: 260-268.

Casaday, G.B. and Hoy, R.R. (1977): Auditory interneurones in the cricket Teleogryllus oceanicus: Physiological and anatomical properties. J. Comp. Physiol. 121: 1-13.

Cooper, J.R., Bloom, F.E. and Roth, R.H. (1982): The biochemical basis of neuropharmacology. 4th Edition. Oxford University Press.

Cokl, A. (1983): Functional properties of vibrorecepters in the legs of Nezara viridula (L.) (Heteroptera, Pentatomidae). J. Comp. Physiol. 150: 261-269.

Davis, N.T. (1982): Improved methods for cobalt filling and silver intensification of insect motor neurones. Stain Technol. 57: 239-244.

Doherty, J.A. (1985a): Trade off phenomenon in calling song recognition and phonotaxis in the cricket, Gryllus bimaculatus (Orthoptera, Gryllidae). J. Comp Physiol. A. 156: 787-801.

Doherty, J.A. (1985b): Temperature coupling and trade-off phenomena in the acoustic communication system of the cricket, Gryllus bimaculatus (DeGeer). J. Exp. Biol. 114: 17-35.

Doolan, J.M. and Pollock, G.S. (1985): Phonotactic specificity of the cricket Teleogryllus oceanicus: intensity dependant selectivity for temporal parameters of the stimulus. J. Comp. Physiol. A. 157: 223-233.

Eibl, E. and Huber, F. (1979): Central projections of tibial sensory fibres within the three thoracic ganglia of the crickets (Gryllus campestris L., Gryllus bimaculatus BeGeer). Zoomorph. 92: 1-17.

Elsner, N. (1983): Insect stridulation and its neurophysiological basis. In Bioacoustics: A Comparative Approach. Ed. B. Lewis. 69-92. Academic Press.

Elsner, N. and Popov, A.V. (1978): Neuroethology of acoustic transmission Adv. Ins. Physiol. 13: 229-355.

Esch, H., Huber, F. and Wohlers, D.W. (1980): Primary auditory neurones in crickets: Physiology and central projections. J. Comp. Physiol. 137: 27-38.

Fielden, A. (1960): Transmission through the last abdominal ganglion of the dragonfly nymph Anax imperator. J. Exp. Biol. 37: 832-844.

Fletcher, N.H. and Hill, K.G. (1978): Acoustics of sound production and of hearing in the bladder cicada Cystosoma saundersii (Westwood) J. Exp. Biol. 72: 43-55.

Gerhardt, H.C. (1975): Sound pressure levels and radiation patterns of the vocalisations of some North American frogs and toads. J. Comp. Physiol. 102: 1-12.

Glautz, R.M. and Kirk, M.D. (1981): Intracellular dye migration and electrotonic coupling with neuronal networks of the crayfish brain. J. Comp. Physiol. A. 140: 120-133.

Gogala, M. (1985a): Vibrational communication in insects (Biophysical and behavioural aspects). In. Acoustic and vibrational communication in insects. Ed. K. Kalmring and N. Elsner. 117-126. Paul Parey.

Gogala, M. (1985b): Vibrational songs of land bugs and their production. In. Acoustic and vibrational communication in insects. Ed. K. Kalmring and N. Elsner. 143-150. Paul Parey.

Gogala, M. and Cokl, A. (1983): The acoustic behaviour of the bug Phymata crassipes (F) (Heteroptera). Rev. Can. Biol. Exptl. 42: 249-256.

Heiligenberg, W. (1969): The effects of stimulus chirps on a crickets chirping (Acheta domesticus). Z. Vergl. Physiol. 65: 70-97.

Helversen, D.v. (1972): Gesang des Mannchens und Lautschema des weibchens bie der Feldheuschnecke. Chorthippus biguttulus (Orthoptera, Acrididae). J. Comp. Physiol. 81: 389-422.

Helversen, D.v. (1984): Parallel processing in auditory pattern recognition and directional analysis by the grasshopper Chorthippus biguttulus L. (Acrididae). J. Comp. Physiol. A. 154: 837-846.

Helversen, D.v. and Helversen, O.v. (1983): Species recognition and acoustic localisation in acridid grasshoppers: a behavioural approach. In. Neuroethology and Behavioural Physiology. Ed. F. Huber and H. Markl. 95-107. Springer-verlag. Heidelberg, Berlin and New York.

Helversen, O.v. and Elsner, N. (1977): The stridulatory movements of acridid grasshoppers recorded with an opto-electric device. J. Comp. Physiol. 122: 53-64.

Hill, K.G. and Boyan, G.S. (1976): Directional hearing in crickets. Nature 262: 390-391.

Hill, K.G. and Boyan, G.S. (1977): Sensitivity to frequency and direction of sound in the auditory system of crickets (Gryllidae). J. Comp. Physiol. 121: 79-97.

Hill, K.G., Loftus-Hills, J.J. and Gartside, D.F. (1972): Pre-mating isolation between the australian field crickets Teleogryllus commodus and T. oceanicus (Orthoptera: Gryllidae). Aust. J. Zool. 20: 153-163.

Hill, K.G. and Oldfield, B.P. (1981): Auditory function in Tettigonioidae. (Orthoptera, Ensifera). J. Comp. Physiol. 142: 169-180.

Hoy, R.R. (1978): Acoustic communication in crickets: a model system for the study of feature detection. Fed. Proc. 37: 2316-2323.

Hoy, R.R. and Paul, R.C. (1973): Genetic control of song specificity in crickets. Science 180: 82-83.

Hutchings, M. and Lewis, B. (1981): Response properties of primary auditory fibres in the cricket Teleogryllus oceanicus (Le Guillou). J. Comp. Physiol. 143: 129-134.

Hutchings, M. and Lewis, B. (1983): Insect sound and vibration receptors. In. Bioacoustics: a Comparative Approach. Ed. Brian Lewis. 181-205. Academic Press.

Hutchings, M. and Lewis, B. (1984): The role of two-tone suppression in song coding by ventral cord neurones in the cricket Teleogryllus oceanicus (Le Guillou). J.Comp. Physiol. 154: 103-112.

Josephson, R.K. and Elder, H.Y. (1968): Rapidly contracting muscles used in sound production by a katydid. Biol. Bull. 135: 409.

Kalmring, K. (1975): The afferent auditory pathway in the ventral cord of Locusta migratoria (Acrididae). II. Responses of the auditory ventral cord neurones to natural sounds. J. Comp Physiol. 104: 143-159.

Kalmring, K. (1983): Convergence of auditory and vibratory senses at the neuronal level of the ventral cord in grasshoppers: its probable importance for behaviour in the habitat. Fortschrite der Zoologie Bd. 28:Horn (Hrsg). Multimodel Convergences in Sensory Systems. Gustav Fischer Verlag. Stuttgart.

Kalmring, K. and Kuhne, K. (1980):The coding of airborne sound and vibration signals in bimodal ventral cord neurones of the grasshopper Tettigonia cantans. J. Comp. Physiol. 139: 267-275.

Kalmring, K., Kuhne, R. and Lewis, B. (1983): The acoustic behaviour of the bushcricket Tettigonia cantans. III. Co-processing of auditory and vibratory information in the central nervous system. Behav. Proc. 8: 213-228.

Keuper, A. and Kuhne, R. 1983. The acoustic behaviour of the bushcricket Tettigonia cantans. II. Transmission of airborne-sound and vibration signals in the biotope. Behav. Proc. 8: 125-145.

Keuper, A., Otto, C., Latimer, W. and Schatral, A. (1985): Airborne sound and vibration signals for bushcrickets at locusts; their importance for the behaviour in the biotope. In Acoustic and Vibrational Communication in Insects. Ed. Kalmring and Elsner. 135-142. Paul Paery.

Kleindienst, H-U., Koch, U.T. and Wohlers, D.W. (1981): Analysis of the cricket auditory system by acoustic stimulation using a closed sound field. J. Comp. Physiol. 141: 283-296.

Koch, U.T. (1980): Analysis of cricket stridulation using miniature angle detectors. J. Comp. Physiol. A. 136: 247-256.

Kramer, E. (1976): The orientation of walking honeybees in odour fields with small concentration gradients. Physiol. Entomol. 1: 27-37

Kuhne, R., Silver, S. and Lewis, B. (1984): Processing of vibratory and acoustic signals by ventral cord neurones in the cricket Gryllus campestris. J. Insect. Physiol. 30: 575-585.

Larsen, O.N. (1981): Mechanical time resolution in some insect ears. II. Impulse sound transmission in acoustic tracheal tubes. J. Comp. Physiol. 143: 297-304.

Larsen, O.N. and Michelsen, A. (1978): Biophysics of the Ensiferan ear. III. The cricket ear as a four-input system. J. Comp. Physiol. 123: 217-227.

Latimer, W.(1980): Song and spacing in Gampsocleis glabra (Orthoptera, Tettigoniidae). J. Natl. Hist. 14: 201-213.

Latimer, W. (1981): The acoustic behaviour of Platycleis albopunotata (Orthoptera, Tettigonidae). Behav. 76: 182-206.

Latimer, W. and Lewis, D.B. (1986): Song harmonic content as a parameter determining acoustic orientation behaviour in the cricket Teleogryllus oceanicus (Le Guillou). J. Comp. Physiol. A. 158: 583-591.

Latimer, W. and Schatral, A. (1983): The acoustic behaviour of the bushcricket Tettigonia cantans. I. Behavioural responses to sound and vibration. Behav. Proc. 8: 113-124.

Lewis, B. 1983. Directional cues for auditory localisation. In Bioacoustics: a Comparative Approach. Ed. Brian Lewis. 233-257. Academic Press.

Littlejohn, M.J. and Harrison, P.A. (1985): The functional significance of the diphasic advertisment call of Goectinia victoriana (Anura: Leptodactylidae) Behav. Ecol. Sociobiol. 16: 363-373.

Loftus-Hills, J.J, Littlejohn, M.J. and Hill, K.G. (1971): Auditory sensitivity of the crickets Teleogryllus oceanicus and T. commodus. Nature (New Biology) 233: 184-185.

Loher, W. and Rence, B. (1978): The mating behaviour of Teleogryllus commodus (Walker) and its central and peripheral control. Z. Tierpsychol 46: 225-259.

Markl, H. (1983): Vibrational communication. In Neuroethology and Behavioural Physiology. Ed. F. Huber and H. Markl. 332-353. Springer-Verlag, Berlin, Heidelberg and New York.

Martens, M.J.M. and Michelsen, A. (1981): Absorption of acoustic energy by plant leaves. J. Acoust. Soc. Am. 69: 303-306.

Marquart, V. (1985): Local interneurones mediating excitation and inhibition onto ascending neurones in the auditory pathway of grasshoppers. Naturwissenschaften 72: 42-44.

Masters, W.M., Tautz, J., Fletcher, N.H. and Markl, H. (1983): Body vibrations and sound production in an insect (Atta sexdens) without specialised radiating structures. J. Comp. Physiol. 150: 239-249.

McKay, J.M. (1969): The auditory system of Homorocoryphus (Tettigoniidae, Orthoptera). J. Exp. Biol. 51: 787-802.

McKay, J.M. (1970): Central control of an insect sensory interneurone. J. Exp. Biol. 53: 137-145.

Michelsen, A. (1983): Biophysical basis of sound communication. In. Bioacoustics: a Comparative Approach. Ed. Brian Lewis. 3-38. Academic Press.

Miller, J.P. and Selverston, A.I. (1979): Rapid killing of single neurones by irradiation of intracellularly injected dye. Science 206: 702-704.

Miller, L.A. (1977): Directional hearing in the locust Schistocerca gregaria Forskal (Acrididae, Orthoptera). J. Comp. Physiol. 119: 85-98.

Miller, L.A. (1983): How insects detect and avoid bats. In Neuroethology and Behavioural Physiology. Ed. Huber and Markl. 251-266. Springer Verlag.

Miller, L.A. and Olesen, J. (1979): Avoidance behaviour in green lacewings. I. Behaviour of free flying green lacewings to hunting bats and ultrasound. J. Comp. Physiol. 131: 113-120.

Moiseff, A. and Hoy, R. (1983): Sensitivity to ultrasound in an identified auditory interneurone in the cricket: a possible neural link to phonotactic behaviour. J. Comp. Physiol. 152: 155-167.

Moiseff, A., Pollack, G.S. and Hoy, R. (1978): Steering responses of flying crickets to sound and ultrasound: mate attraction and predator avoidance. Proc. Natl. Acad. Sci. USA 75: 4052-4056.

Morris, G.K. (1971): Aggression in male conocephalic grasshoppers (Tettigoniidae). Anim. Behav. 19: 132-137.

Narins, P.M. and Capranica, R.R. (1976): Sexual differences in the auditory system of the tree frog Eleutherodactylus coqui. Science. 192: 378-380.

Nocke, H. (1971): Biophysik der schallerzeugung durch die Vorderfluger der Grillen Z. Vergl. Physiol. 74: 272-314.

Nocke, H. (1972): Physiological aspects of sound communication in crickets (Gryllus campestris L.) J. Comp. Physiol. 80: 141-162.

Nolen, T.G. and Hoy, R.R (1984): Initiation of behaviour by single neurones: The role of behavioural context. Science 226: 992-994.

Nolen, T.G. and Hoy, R.R. (1986a): Phonotaxis in flying crickets I. Attraction to the calling song and avoidance of bat-like ultrasound are discrete behaviours. J. Comp. Physiol. A. 159: 423-439.

Nolen, T.G. and Hoy, R.R (1986b): Phonotaxis in flying crickets. II. Physiological mechanisms of two-tone suppression of the high frequency avoidance steering behaviour by the calling song. J. Comp. Physiol. A. 159: 441-456.

Oldfield, B.P. (1980): Accuracy of orientation in female crickets, Teleogryllus oceanicus (Gryllidae): Dependance on song spectrum. J. Comp. Physiol. A. 141: 93-99.

Oldfield, B.P. (1982): Tonotopic organisation of auditory receptors in Tettigoniidae (Orthoptera: Ensifera). J. Comp. Physiol. A. 147: 461-469.

Oldfield, B.P. (1985): The tuning of auditory receptors in bushcrickets. Hear. Res. 17: 27-35.

Oldfield, B.P. and Hill, K.G. (1983): The physiology of ascending auditory interneurones in the Tettigoniid Caedicia simplex (Orthoptera, Ensifera). J. Comp. Physiol. A. 152: 495-508.

Oldfield, B.P., Kleindienst, H-U. and Huber, F. (1986): Physiology and tonotopic organisation of auditory receptors in the cricket Gryllus bimaculatus DeGeer. J. Comp. Physiol. A. 159: 457-461.

Pearson, K. and Fourtner, C.R. (1975): Nonspiking interneurones in the walking system of the cockroach. J. Neurophysiol. 38: 155-167.

Pollack, G.S. (1982): Sexual differences in cricket calling song recognition. J. Comp. Physiol. 146: 217-221.

Pollack, G.S. (1983): Ultrasound-sensitive neurones descending in the thoracic nervous system of the cricket Teleogryllus oceanicus. Can. J. Zool. 62: 555-562.

Pollack, G.S. (1986): Discrimination of calling song models by the cricket Teleogryllus oceanicus: the influence of sound direction on neural encoding of the stimulus temporal pattern and on phonotactic behaviour. J. Comp. Physiol. A. 158: 549-561.

Pollack, G.S. and Hoy, R.R. (1979): Temporal pattern as a cue for species specific calling song recognition in crickets. Science 204: 429-432.

Pollack, G.S. and Hoy, R. (1981): Phonotaxis to individual rythmic components of a complex cricket calling song. J. Comp. Physiol. 144: 367-373.

Pollack, G.S., Huber, F and Weber, T. (1984): Frequency and temporal pattern dependant phonotaxis of crickets Teleogryllus oceanicus during tethered flight and compensated walking. J. Comp. Physiol. A. 154: 13-26.

Popov, A.V. (1981): Sound production and hearing in the cicada, Cicadetta sinuatipennis Osh. (Homoptera, Cicadidae). J. Comp. Physiol. 142: 271-280.

Popov, A.V. and Markovich, A.M. (1982): Auditory interneurones in the prothoracic ganglion of the cricket Gryllus bimaculatus. II. A high frequency ascending neurone (HF1AN). J. Comp. Physiol. 146: 351-359.

Popov, A.V., Markovich, A.M. and Andjan, A.S. (1978): Auditory interneurones in the prothoracic ganglion of the cricket Gryllus bimaculatus DeGeer. I. The large segmental auditory neurone (LSAN). J. Comp. Physiol. 126: 183-192.

Popov, A.V. and Shuvalov, V.F. (1977): Phonotactic behaviour of crickets. J. Comp. Physiol. 119: 111-126.

Prozesky-Schulze, L., Prozesky, O.P.M., Anderson, F. and van der Merwe, G.J.J. (1975): Use of a self-made baffle by a tree cricket. Nature 255: 142-143.

Rehbein, H., Kalmring, K. and Romer, H. (1974): Structure and function of acoustic neurones in the thoracic ventral nerve cord of Locusta migratoria (Acrididae). J. Comp. Physiol. 95: 263-280.

Rheinlaender, J., Kalmring, K., Popov, A.V. and Rehbein, H. (1976): Brain projections and information processing of biologically significant sounds by two large ventral cord neurones of Gryllus bimaculatus DeGeer (Orthoptera, Gryllidae). J. Comp. Physiol. 110: 251-269.

Roeder, K.D. (1967): Nerve Cells and Insect Behaviour. Harvard Press, Cambridge, Mass.

Romer, H. (1983): Tonotopic organisation of the auditory neuropile in the bushcricket Tettigoniia viridissima. Nature 306: 60-62.

Romer, H. and Marquart, V. (1984): Morphology and physiology of auditory interneurones in the metathoracic ganglion of the locust. J. Comp. Physiol. 155: 249-262.

Romer, H. and Seikowski, U. (1985): Responses to model songs of auditory neurones in the thoracic ganglia and brain of the locust. J. Comp. Physiol. 156: 845-860.

Rose, G.J. and Capranica, R.R. (1984): Processing amplitude modualted sounds by the auditory midbrain of two species of toads: matched temporal filters. J. Comp. Physiol. 154: 211-219.

Sales, G.D. and Pye, J.D. 1974. Ultrasonic Communication by Animals. Chapman and Hall, London.

Samways, M.J. (1977): Bushcricket interspecific acoustic interactions in the field (Orthoptera, Tettigoniidae). J. Nat. Hist. 11: 155-168.

Schatral, A., Latimer, W. and Broughton, W.B. (1984): Spatial dispersion and agonistic contacts of male bushcrickets in the biotope. Z. Tierpsychol. 65: 201-214.

Schatral, A., Latimer, W. and Kalmring, K. (1985): The role of the song for spatial dispersion and agonistic contacts in male bushcrickets. In. Acoustic and Vibrational Communication in Insects. Ed. K. Kalmring and N. Elsner. 111-116. Paul Parey.

Schildberger, K. (1984): Temporal selectivity of identified auditory neurones in the cricket brain. J. Comp. Physiol. A. 155: 171-185.

Schildberger, K. (1985): In Acoustic and Vibrational Communication in Insects. 41-52. Ed. Kalmring and Elsner. Paul Paery.

Selverston, A.I., Kleindienst, H. and Huber, F. (1985): Synaptic connectivity between cricket auditory interneurones as studied by selective photoinactivation. J. Neurosci. 5: 1283-1292.

Silver, S.C., Kuhne, R. and Lewis, D.B. (1984): Two-tone interactions and song coding in identified ventral cord neurones in the cricket. Acoustic Letters 7: 135-140.

Simmons, P.J. (1977): Neuronal generation of singing in a cicada. Nature. 270: 243-245.

Simmons, P. (1982): The operation of connections between photoreceptors and large second order neurones in dragonfly ocelli. J. Comp. Physiol. 146: 389-398.

Simmons, P.J. and Young, D. (1978): The tymbal mechanism and song patterns of the bladder cicada Cystosoma saundersii. J. Exp. Biol. 76: 27-45.

Sismondo, E. (1979): Stridulation and tegminal resonance in the tree cricket Oecanthus nigricornis (Orthoptera, Gryllidae. Oecanthinae). J. Comp. Physiol. 129: 269-279.

Skovmand, O. and Pedersen, S.B. (1978): Tooth impact rate in the song of a shorthorned grasshopper: a parameter carring specific behavioural information. J. Comp. Physiol. 124: 27-36.

Stewart, W.W. (1978): Functional connections between cells as revealed by dye-coupling with a highly fluorescent napthalimide tracer. Cell 14: 741:759.

Stewart, W.W. (1981): Lucifer dyes: highly fluorescent dyes for biological tracing. Nature 292: 17-21.

Stout, J.F. and Huber, F. (1972): Responses of central auditory neurones of female crickets (Gryllus campestris L.) to the calling song of the male. Z. Verg. Physiol. 76: 302-313.

Stout, J.F. and Huber, F. (1981): Responses to features of the calling song by ascending auditory interneurones in the cricket Gryllus campestris. Physiol. Entomol. 6: 199-212.

Stout, J.F. and Weber, T. (1981): Visual influences on phonotactic orientation to model calling songs by crickets: a comparison of techniques. Am. Zool. 21: 960.

Stout, J.F., Atkins, G. and Burghardt, F. (1985): The characteristics and possible importance for phonotaxis of L-shaped ascending acoustic interneurones in the cricket (Acheta domesticus). In Acoustic and Vibrational Communication in Insects. Ed. Kalmring and Elsner. Paul Paery.

Stout, J.F., DeHaan, C.H. and McGhee, R.W. (1983): Attractiveness of male Acheta domesticus calling song to females. I. Dependance on each of the calling song features. J. Comp. Physiol. 153: 509-521.

Suga, N. and Katsuki, Y. (1961): Pharmacological studies on the auditory synapses in a grasshopper. J. Exp. Biol. 38: 759-770.

Taylor, M. (1978): Trapeziod waveform generator. New. Electronics 11: 20-21.

Thorsen, J., Weber, T. and Huber, F. (1982): Auditory behaviour of the cricket. II. Simplicity of calling song recognition in Gryllus and anomalous phonotaxis at abnormal carrier frequencies. J. Comp. Physiol. 146: 361-378.

Timm, F. (1958): Zur histologie der schwermetalle. Das sulfid-silbervartahren. Deutche Zeitschrift der Gesellschalt fur Gerichtliche Medizin. 46: 706-711.

Tschinkel, W.A. and Doyan, J.T. (1976): Sound production by substral tapping in beetles of the genus Eusattus (Tettyriidae, Coniontini). Coleop. Bull. 30: 331-335.

Tucker, V.A. (1969): Wavemaking by whirligig beetles (Gyrinidae). Science. 166: 897-899.

Ulagaraj, S.M. and Walker, T.J. (1973): Phonotaxis of crickets in flight: attraction of male and female crickets to male calling songs. Science 182: 1278-1279.

Ulagaraj, S.M. and Walker, T.J. (1975): Response of flying mole crickets to three parameters of synthetic songs broadcast outdoors. Nature 253: 530-531.

Walker, T.J. (1957): Specificity in the response of female tree crickets (Orthoptera, Gryllidae, Oecanthinae) to calling songs of the males. Anal. Ent. Soc. Am. 50: 626-636.

Walker, T.J. and Dew, D. (1972): Wing movements of calling katydids: fidding finese. Science, New York. 178: 174-176.

Walker, T.J. (1969): Acoustic synchrony. Two mechanisms in the snowy tree cricket. Science 166: 891-894.

Weber, T. (1984): Acoustical pattern recognition in crickets. In Localisation and Orientation in Biology and Engineering. 181-185. Ed. Varju and Schnitzler. Springer Verlag.

Weber, T., Thorsen, J. and Huber, F. (1981): Auditory behaviour of the cricket. I. Dynamics of compensated walking and discrimination paradigms on the Kramer treadmill. J. Comp. Physiol. 141: 215-232.

Wiese, K. and Eilts, K. (1985): Evidence for matched frequency dependance of bilateral inhibition in the auditory pathway of Gryllus bimaculatus. Zool. Jb. Physiol. 89: 181-201.

Wiese, K. and Eilts-Grimm, K. (1985): Functional potential of recurrent lateral inhibition in cricket audition. In Acoustic and Vibrational communication in insects. 33-40. Ed. Kalmring and Elsner. Paul Parey.

Wohlers, D.W. and Huber, F. (1978): Intracellular recording and staining of cricket auditory interneurones (Gryllus campestris L., Gryllus bimaculatus DeGeer) J. Comp. Physiol. 127: 11-29.

Wohlers, D.W. and Huber, F. (1982): Processing of sound signals by six types of neurones in the prothoracic ganglion of the cricket, Gryllus bimaculatus L. J. Comp. Physiol. 146: 161-173.

Wohlers, D.W and Huber, H. (1985): Topographical organisation of the auditory pathway within the prothoracic ganglion of the cricket Gryllus bimaculatus. L. Cell Tissue Res. 239: 555-565.

Zaretsky, M.D. (1972): Specificity of the calling song and short term changes in the phonotactic response by female crickets, Scapsipedus marginatus (Gryllidae). J. Comp. Physiol. 79: 153-172.

FIELDEN'S RINGER

NaCl	- 7.5g	
KCl	- 0.1g	
CaCl	- 0.2g	
NaHCO	- 0.2g	in 1000ml at pH7.

REPLAY PROGRAM TO PLOT THRESHOLD CURVES

```
>L.
  10REM PROGRAM TO PLOT THRESHOLD CURVES FROM CO-ORDINATES STORED IN
  20REM DATA FILES ON DISK.
  30REM BGH/LAH  LAST MOD.  15-APR-85.
  40@%=10:NX%=8:XMIN=100:XMAX=1000:XSCL=(XMAX-XMIN)/2:YMIN=50:YMAX=900
  50DIM X%(40),Y%(40)
  60MODE 0
  80VDU 28,0,1,79,0
  90CLS:CLG
 100PRINT"ENSURE SECOND PROCESSOR OFF AND *SD"
 110 PROCreadfile
 120PROCprintout
 130PROCaxes
 140PROCplotout
 150INPUT "NEXT ACTION?   end <RET>   NEWPLOT <1>   NEW AXES <2> ":0%
 160 IF 0%=1 THEN 110
 170IF 0%=2 THEN 90
 180INPUT"SCREEN DUMP<Y/n>":0$
 190CLS:VDU26:PRINT IDENT$:MOVE XMAX-100,YMAX+40:VDU5:PRINT FILE$
 200MOVE XMAX-100,YMAX:PRINT"L ":A%=40:X%(A%)=XMAX-50:Y%(A%)=YMAX-12:
PROCosquare
 210MOVE XMAX-100,YMAX-40:PRINT"R ":A%=39:X%(A%)=XMAX-50:Y%(A%)=
YMAX-52:PROCcsquare:VDU4
 220IF 0$<>"N" THEN CALL&900
 230PRINT "END"
 240END
 250
 260
 270DEF PROCprintout
 280 INPUT "PRINT OUT TABLE (y/N)":0$
 290IF 0$<>"Y" THEN 410
 300VDU2
 310PRINT IDENT$
 320PRINT SPC(1)
 325@%=&205
 330PRINT"   NO.        FR.(kHz)   ATTEN(dBSPL)"
 340FOR A%=1 TO N%
 350REM CONVERT GRAFIX UNITS TO SCALE UNITS.
 360XVAL=(X%(A%)-XMIN)/XSCL:XVAL=10^XVAL
 370YVAL=((Y%(A%)-YMIN)*80/(YMAX-YMIN))+20
 380PRINT  A%;"      ",XVAL"     ",YVAL
 390NEXT
 395@%=10
 400VDU3:CLS
 410ENDPROC
 420
 430
 440DEF PROCplotout
 442INPUT"DEFAULT SYMBOLS?(Y/n)":0$
 443IF 0$<>"N" THEN PROCdfsymbols:GOTO 530
 450INPUT "CROSSES, OPEN SQUARES OR CLOSED SQUARES.  (<RET>/1/2)":0%
 460 FOR A%=1 TO N%
 470IF A%=1 MOVE X%(A%),Y%(A%) ELSE DRAWX%(A%),Y%(A%)
 480IF 0%=0 THEN PROCcross
 490    IF 0%=1 THEN PROCosquare
 500IF 0%=2 THEN PROCcsquare
 510IF 0%<0 OR 0%>2 THEN PRINT " SILLY SOD!  TRY AGAIN":GOTO 450
 520NEXT
 530ENDPROC
 540
 550
 560DEF PROCaxes
 570LOCAL X%,Y%
 580MARGX%=90:MARGY%=10:OFSET%=70
```

```
530ENDPROC
540
550
560DEF PROCaxes
570LOCAL X%,Y%
580MARGX%=90:MARGY%=10:OFSET%=70
590XPOS=XMIN+(XSCL*LOG(2))
600MOVE XPOS,YMIN
610LOW%=2:HIGH%=10:INC%=1:MARK%=20
620FOR X%=LOW% TO HIGH% STEP INC%
630LGX=LOG(X%)
640XPOS=XMIN+(XSCL*LGX)
650DRAW XPOS,YMIN
660DRAW XPOS,MARK%+YMIN
670MOVE XPOS-150,YMIN-MARGY%
680VDU 5
690IF X%>5 AND X%<10 THEN PRINT:SPC(1) ELSE PRINT X%
700MOVE XPOS,YMIN
710NEXT
720IF X%>=10 AND X%<=50 THEN 740
730IF X%>=50 AND X%<100 THEN 760 ELSE 780
740LOW%=20:HIGH%=50:INC%=10
750GOTO 620
760LOW%=50:HIGH%=100:INC%=50
770GOTO 620
780XMIN=XMIN+OFSET%
790MOVE XMIN,YMIN
800LOW%=20:HIGH%=100:INC%=20
810YPOS=YMIN
820YSCL=(YMAX-YMIN)/4
830FOR Y%=LOW% TO HIGH% STEP INC%
840DRAW XMIN,YPOS
850DRAW XMIN+MARK%,YPOS
860MOVE XMIN-MARGX%,YPOS
870MOVE -MARGX%+OFSET%,YPOS
880PRINT Y%
890MOVE XMIN,YPOS
900YPOS =YPOS+YSCL
910NEXT
920MOVE XMAX-100,YMIN+50: PRINT "F(kHz)"
930MOVE XMIN,YMAX+40: PRINT "dB(SPL)"
940VDU4
950XMIN=XMIN-OFSET%
960ENDPROC
970
980
990DEF PROCcross
1000MOVE X%(A%)+NX%,Y%(A%)+NX%
1010DRAW X%(A%)-NX%,Y%(A%)-NX%
1020MOVE X%(A%)-NX%,Y%(A%)+NX%
1030DRAW X%(A%)+NX%,Y%(A%)-NX%
1040 MOVE X%(A%),Y%(A%)
1050 ENDPROC
1060
1070
1080  DEF PROCcsquare
1090MOVE X%(A%)+NX%,Y%(A%)+NX%
1100FOR NX%=8 TO 2 STEP-2
1110PROCosquare
1120NEXT
1130MOVE X%(A%),Y%(A%)
1140NX%=8
1150ENDPROC
1160
1170DEF PROCosquare
1180MOVE X%(A%)+NX%,Y%(A%)+NX%
1190DRAW X%(A%)-NX%,Y%(A%)+NX%
1200DRAW X%(A%)-NX%,Y%(A%)-NX%
1210DRAW X%(A%)+NX%,Y%(A%)-NX%
```

```
1190DRAW X%(A%)-NX%,Y%(A%)+NX%
1200DRAW X%(A%)-NX%,Y%(A%)-NX%
1210DRAW X%(A%)+NX%,Y%(A%)-NX%
1220DRAW X%(A%)+NX%,Y%(A%)+NX%
1230MOVE X%(A%),Y%(A%)
1240ENDPROC
1245
1246
1250DEF PROCreadfile
1260INPUT "FILENAME";FILE$
1270Z=OPENIN FILE$
1280INPUT£Z,IDENT$
1285INPUT£Z,A$
1290PRINT IDENT$
1300N%=0
1310REPEAT
1320N%=N%+1
1330INPUT£Z,X$,Y$
1340X%(N%)=VAL(X$)
1350Y%(N%)=VAL(Y$)
1360UNTIL EOF£Z
1370CLOSE£Z
1380ENDPROC
1385
1386
1390DEF PROCdfsymbols
1392FOR A%=1 TO N%
1393IF A%=1 MOVE X%(A%),Y%(A%) ELSE DRAWX%(A%),Y%(A%)
1400IF A$="R" THEN PROCcsquare
1410IF A$="L" THEN PROCosquare
1415NEXT
1420ENDPROC
```

SONG PRODUCTION PROGRAM

```
>L.
  10 MODE 0
  20DIM X% 20:Y%=X% DIV 256:OSWORD=&FFF1
  30 REM "TOSONGS"
  40 REM LAST MOD. 25.3.85 B.G.H., L.A.H.
  50 REM HITTING "RETURN" DURING MENU INPUTS PREVIOUS PARAMETERS.
  60 REM Hit SPACE to end a song cycle.
  70STYPE%=1:RANGE=0:F=18:QSET%=0
  80PROCwriteIO(0,&FCC2)
  90PROCwriteIO(0,&FCC3):PROCwriteIO(3,&FCC1)
 100 CLS:CYCLE%=0
 110IF QSET%<>0 THEN 150
 120INPUT "TWO TONES ? <Y/n>";Q$
 130QSET%=1
 140IF Q$="N" THEN AVALU%=2 ELSE AVALU%=6
 150INPUT "SEQUENCE,COURTSHIP,CALL,AGGRESSION, TRAIN (0,1,2,3,4)":X
 160 STYPE%=X
 170IF STYPE%=0 THEN PERIOD%=200:DELAY%=20
 180 IF STYPE%=1 THEN PERIOD%=250:DELAY%=20
 190 IF STYPE%=2 THEN PERIOD%=145:DELAY%=1
 200IF STYPE%=3 THEN PERIOD%=250:DELAY%=10
 210 IF STYPE%=4 THEN PERIOD%=250:DELAY%=20
 220IF STYPE%=4 THEN INPUT "CHP VALUE (3-10)";CHP
 230PROCsetfr
 240 REM RETURN TO MENU IF "SPACE"
 250 REPEAT
 260IF INKEY(1)=32 THEN 100
 270 REM SET UP TRIG TIMES
 280T=TIME:TD=T+DELAY%:T1=T+1:T2=T+2:TP=T+PERIOD%
 290 REM TRIGGER THE SCOPE
 300REPEAT UNTIL TIME>=T1:PROCwriteIO(17,&FCC2):REPEAT UNTIL
TIME>=T2:PROCwriteIO(0,&FCC2)
 310 IF STYPE%=0 THEN PROCsequence
 320 IF STYPE%=1 THEN PROCcourt
 330 IF STYPE%=2 THEN PROCcall
 340 IF STYPE%=3 THEN PROCaggr
 350 IF STYPE%=4 THEN PROCtrain
 360REPEAT UNTIL TIME>=TP
 370 UNTIL FALSE
 380END
 390
 400
 410 DEF PROCcourt
 420 REPEAT UNTIL TIME>=TD
 430SUM=TD
 440 SUM1=SUM+1
 450CHP=8
 460PROCwriteIO(AVALU%,&FCC2)
 470 REPEAT UNTIL TIME>=SUM1
 480PROCwriteIO(0,&FCC2)
 490FOR N=1 TO 3
 500 PROCpulse
 510 NEXT N
 520 REPEAT UNTIL TIME>=SUM+3
 530PROCwriteIO(0,&FCC1)
 540CHP=6
 550 PROCpulse
 560FOR N=1 TO 40
 570CHP=3
 580 PROCpulse
 590NEXT
 600 REPEAT UNTIL TIME>=SUM+3
 610PROCwriteIO(3,&FCC1)
 620ENDPROC
```

```
570CHP=3
580 PROCpulse
590NEXT
600 REPEAT UNTIL TIME>=SUM+3
610PROCwriteIO(3,&FCC1)
620ENDPROC
630
640
650 DEF PROCcall
660SUM=TD
670CHP=5
680FOR N=1 TO 4
690 PROCpulse
700 NEXT N
710CHP=9
720 PROCpulse
730FOR N=1 TO 7
740CHP=4
750 PROCpulse
760CHP=11
770 PROCpulse
780NEXT
790 CHP=4
800 PROCpulse
810ENDPROC
820
830
840 DEF PROCaggr
850SUM=TD
860CHP=6
870FOR N=1 TO 16
880 PROCpulse
890 NEXT N
900CHP=22
910 PROCpulse
920CHP=4
930 PROCpulse
940 ENDPROC
950
960
970 DEF PROCsetfr
980 INPUT "FREQUENCY (2-20/20-200KHz.) ";X
990 IF X =0 THEN 1010
1000 F=X
1010 IF F<2 OR F>200 THEN GOTO 1110
1020 IF F>=20.01 THEN F=F/10:RANGE=1
1030 VCO%=(F-2)*255/18
1040 OUTFR=(VCO%*18/255)+2
1050 IF RANGE>0 THEN OUTFR=OUTFR*10
1060 @%=&20205
1070 PRINT TAB(55,0) "FREQUENCY = ca.";OUTFR;" kHz"
1080 @%=10
1090PROCwriteIO(VCO%,&FCC3)
1100 GOTO 1130
1110 PRINT "ERROR ---OUT OF FREQUENCY RANGE ."
1120 GOTO 980
1130 ENDPROC
1140
1150 DEF PROCpulse
1160 SUM=SUM+CHP:SUM1=SUM+1
1170REPEAT UNTIL TIME>=SUM:PROCwriteIO(AVALU%,&FCC2):
REPEAT UNTIL TIME>=SUM1:PROCwriteIO(0,&FCC2)
1180 ENDPROC
1190
1200DEF PROCsequence
1210CYCLE%=CYCLE%+1
1220IF CYCLE%=1 THEN PROCcourt
1230IF CYCLE%=2 THEN PROCcall
1240IF CYCLE%=3 THEN PROCaggr
```

```
1210CYCLE%=CYCLE%+1
1220IF CYCLE%=1 THEN PROCcourt
1230IF CYCLE%=2 THEN PROCcall
1240IF CYCLE%=3 THEN PROCaggr
1250IF CYCLE%=3 THEN CYCLE%=0
1260ENDPROC
1270
1280DEF PROCtrain
1290REPEAT UNTIL TIME>=TD
1300SUM=TD
1310SUM1=SUM+1
1320FOR N=1 TO 40
1330PROCpulse
1340NEXT
1350REPEAT UNTIL TIME>=SUM+3
1360ENDPROC
1370
1380DEF PROCwriteIO(data,addr):!X%=addr:X%?4=data
1390A%=6:CALL OSWORD:ENDPROC
```

PROGRAM TO TRIGGER TONE AND CURRENT PULSES, FREQUENCY GENERATION AND PLOT THRESHOLD CURVES.

```
L.
  10REM BGH/LAH 1985  LAST MOD. 24/4/85
  20REM TOG CONTROLS TWO OSCILATORS AND CURRENT INJECTION.
  30DIM X% 20: Y%=X% DIV 256 : OSWORD=&FFF1
  40REM ALLOCATE MEMORY FOR WRITING TO INTERBEEB WHEN 2ND PROCESSOR ON.
  50MODE 0
  60CLS
  70DIM PTS(40,2),DB(23,3)
  80DATA 2,3,4,4,5,5,6,8,10,12,14,16,18,20,25,30,35,40,50,60,70,80,90,
  100
  90DATA 106,109,116,114,113,110,110,113,108,111,110,113,113,116,115,
  112,108,102,96,96,89,68,68
 100DATA 97,101,112,112,111,101,110,108,109,104,109,102,105,110,114,
,99,86,0,0,0,0,0,0
 110Q%=1
 120REPEAT
 130REMREAD CALL CURVES INTO ARRAY
 140FOR N%=1 TO 23
 150READ DB(N%,Q%)
 160NEXT
 170Q%=Q%+1
 180UNTILQ%=4
 190VDU 28,0,1,49,0
 200PROCaxes
 210INPUT "FIXED PERIOD 500ms ? (Y/n)";Q$
 220INPUT "STANDARD DELAYS - NO SECOND TONE - <Y/n>";QU$
 230ACOUNT=0:NX=6:DI%=2:DT1%=2:DT2%=-1:F=10:PRD=.5
 240ZZ%=0
 250RANGE=0:ARROW%=0
 260PROCwriteIO(3,&FCC1):PROCwriteIO(0,&FCC2):PROCwriteIO(0,&FCC3)
 270CLS
 280PROCsetfr
 290PROCtones
 300GOTO 250
 310END
 320
 330DEF PROCsetfr
 340INPUT "FREQUENCY (2-20/20-200KHz.) ";X
 350IF X =0 THEN 370
 360F=X
 370IF F<2 OR F>200 THEN PRINT "ERROR ---OUT OF FREQUENCY RANGE ."
 :GOTO 340
 380IF F>=20.01 THEN F=F/10:RANGE=1
 390VCO%=(F-2)*255/18
 395REM CALCULATE FRED VALUE TO PRODUCE REQUIRED FR.
 400OUTFR=(VCO%*18/255)+2
 410IF RANGE>0 THEN OUTFR=OUTFR*10
 420VDU5:MOVE 1200,1023
 430REM                          ----------ERASE LAST OUTFR----------
 440FOR N%=1 TO 10
 450VDU127
 460NEXT
 470REM                                    ----------------
 480@%=&20205
 490MOVE 800,1023:PRINT "FREQUENCY = ca.";OUTFR" kHz":VDU4
 500@%=10
 510PROCwriteIO(VCO%,&FCC3)
 520GOTO 530
 530ENDPROC
 540
 550DEF PROCtones
 560IF Q$<>"N" THEN 600
```

```
510PROCwriteIO(VLO%,&FCC3)
520GOTO 530
530ENDPROC
540
550DEF PROCtones
560IF Q$<>"N" THEN 600
570INPUT"CYCLE PERIOD ?<SECS>";X
580IF X=0 THEN 600
590PRD=X
600GH%=DT1%*10:LH%=DT2%*10
610IF QU$<>"N" THEN Y=0:Y1=0:Y2=0:GOTO 670
620IF DT1%<0 THEN GH%=-1
630IF DT2%<0 THEN LH%=-1
640MOVE 400,990:VDU5:PRINT GH%",";LH%",";DI%*10:VDU4
650REM IF Y VALUES ARE +VE THEN DELAYS ARE RESET, IF -VE TRIGGERS
 ARE SWITCHED OFF. IF 0 THE DEFAULT OR PREVIOUS VALUES FOR DELAYS
 ARE USED
660INPUT"DELAYS FOR  T1, T2, Iinj. <MSEC>";Y1,Y2,Y
670IF Y>0 THEN DI%=INT(Y/10)
680IF Y1>0 THEN DT1%=INT(Y1/10)
690IF Y2>0 THEN DT2%=INT(Y2/10)
700IF Y1<0 THEN DT1%=Y1
710IF Y2<0 THEN DT2%=Y2
720IF DI%=0 OR DT1%=0 OR DT2%=0 THEN PRINT"INCORRECT DELAYS":GOTO 660
730Q%=4
740REM K%,L%,M% STORES VALUES FOR FRED2.
750IF DT1%=DT2% AND DT1%=DI% THEN Q%=0:K%=14
760IF DT1%=DT2% AND DT1%<>DI% THEN Q%=1:K%=6:L%=8
770IF DT1%=DI% AND DT1%<>DT2% THEN Q%=2:K%=4:L%=10
780IF DT2%=DI% AND DT2%<>DT1% THEN Q%=3:K%=12:L%=2
790IF Y1<0 THEN Q%=5:K%=4:L%=8:M%=12
800IF Y2<0 THEN Q%=6:K%=2:L%=8:M%=10
810IF Y1<0 AND Y2<0 THEN Q%=7:K%=8
820PRD%=PRD*100
830BB%=0
840REPEAT
850IF INKEY(1)=32 THEN BB%=1
860IF ARROW%=1 THEN 1020
870A%=(TIME+2):B%=(A%+1):C%=(A%+DT1%):D%=(A%+DT2%):E%=(A%+DI%):
F%=(A%+PRD%-2)
880REM TRIGGER THE SCOPE.
890REPEAT UNTIL TIME>=A%
900PROCwriteIO(17,&FCC2)
910REPEAT UNTIL TIME>=B%
920PROCwriteIO(0,&FCC2)
930IF Q%=0 THEN PROCzero(C%,K%)
940IF Q%=1 THEN PROCone(C%,E%,K%,L%)
950IF Q%=2 OR Q%=3 THEN PROCone(D%,C%,K%,L%)
960IF Q%=4 THEN PROCtwo
970IF Q%=5 THEN PROCthree(D%,E%,K%,L%)
980IF Q%=6 THEN PROCthree(C%,E%,K%,L%)
990IF Q%=7 THEN PROCzero(E%,K%)
1000UNTIL BB%=1
1010PROCplot
1020ENDPROC
1030
1040DEF PROCzero(G%,I%)
1050REPEAT
1060IF TIME=G% THEN PROCwriteIO(I%,&FCC2):REPEAT UNTIL TIME>G%+1:
PROCwriteIO(0,&FCC2)
1070UNTIL TIME=F%
1080ENDPROC
1090REM SIMULTANEOUS OUTPUTS OR CURRENT ONLY.
1100
1110DEF PROCone(G%,H%,I%,J%)
1120REPEAT
1130IF TIME=G% THEN PROCwriteIO(I%,&FCC2):REPEAT UNTIL TIME>=G%+1:
PROCwriteIO(0,&FCC2)
1140IF TIME=H% THEN PROCwriteIO(J%,&FCC2):REPEAT UNTIL TIME>=H%+1:
```

```
1130IF TIME=G% THEN PROCwriteIO(I%,&FCC2):REPEAT UNTIL TIME>=G%+1:
PROCwriteIO(0,&FCC2)
1140IF TIME=H% THEN PROCwriteIO(J%,&FCC2):REPEAT UNTIL TIME>=H%+1:
PROCwriteIO(0,&FCC2)
1150UNTIL TIME=F%
1160ENDPROC
1170REM TWO OUT OF THREE EVENTS SIMULTANEOUS
1180
1190DEF PROCtwo
1200REPEAT
1210IF TIME=C% THEN PROCwriteIO(2,&FCC2):REPEAT UNTIL TIME=C%+1:
PROCwriteIO(0,&FCC2)
1220IF TIME=D% THEN PROCwriteIO(4,&FCC2):REPEAT UNTIL TIME=D%+1:
PROCwriteIO(0,&FCC2)
1230IF TIME=E% THEN PROCwriteIO(8,&FCC2):REPEAT UNTIL TIME=E%+1:
PROCwriteIO(0,&FCC2)
1240UNTIL TIME=F%
1250ENDPROC
1260REM ALL DIFFERENT
1270
1280DEF PROCthree(G%,H%,I%,J%)
1290IF G%=H% THEN 1350
1300REPEAT
1310IF TIME=G% THEN PROCwriteIO(I%,&FCC2):REPEAT UNTIL TIME>=G%+1:
PROCwriteIO(0,&FCC2)
1320IF TIME=H% THEN PROCwriteIO(J%,&FCC2):REPEAT UNTIL TIME>=H%+1:
PROCwriteIO(0,&FCC2)
1330UNTIL TIME>=F%
1340GOTO 1380
1350REPEAT
1360IF TIME=G% THEN PROCwriteIO(M%,&FCC2):REPEAT UNTIL TIME>=G%+1:
PROCwriteIO(0,&FCC2)
1370UNTIL TIME>=F%
1380ENDPROC
1390REM CURRENT+ONE TONE
1400
1410DEF PROCaxes
1420XMIN=100:XMAX=1000:XSCL=(XMAX-XMIN)/2:YMIN=50:YMAX=900
1430MARGX%=90:MARGY%=10
1440XPOS=XMIN+(XSCL*LOG(2))
1450MOVE XPOS,YMIN
1460REM PLOT X AXIS
1470LOW%=2:HIGH%=10:INC%=1:MARK%=20
1480FOR WX%=LOW% TO HIGH% STEP INC%
1490LGX=LOG(WX%)
1500XPOS=XMIN+(XSCL*LGX)
1510DRAW XPOS,YMIN
1520DRAW XPOS,MARK%+YMIN
1530MOVE XPOS-150,YMIN-MARGY%
1540VDU 5
1550IF WX%>5 AND WX%<10 THEN PRINT;SPC(1) ELSE PRINT WX%
1560MOVE XPOS,YMIN
1570NEXT
1580IF WX%>=10 AND WX%<=50 THEN 1600
15901F WX%>=50 AND WX%<100 THEN 1620 ELSE 1650
1600LOW%=20:HIGH%=50:INC%=10
1610GOTO 1480
1620LOW%=50:HIGH%=100:INC%=50
1630GOTO 1480
1640REM PLOT Y AXIS
16500FSET%=50:XMIN=XMIN+OFSET%
1660MOVE XMIN,YMIN
1670LOW%=20:HIGH%=100:INC%=20
1680YPOS=YMIN
1690YSCL=(YMAX-YMIN)/4
1700FOR WY%=LOW% TO HIGH% STEP INC%
1710DRAW XMIN,YPOS
1720DRAW XMIN+MARK%,YPOS
1730MOVE XMIN-MARGY%,YPOS
```

```
1710DRAW XMIN,YPOS
1720DRAW XMIN+MARK%,YPOS
1730MOVE XMIN-MARGX%,YPOS
1740MOVE -MARGX%+OFSET%,YPOS
1750PRINT WY%
1760MOVE XMIN,YPOS
1770YPOS =YPOS+YSCL
1780NEXT
1790MOVE XMAX-100,YMIN+50: PRINT "F(kHz)"
1800MOVE XMIN,YMAX+40: PRINT "dB(SPL)"
1810VDU4
1820XMIN=XMIN-OFSET%
1830ENDPROC
1840
1850DEF PROCplot
1860IF ZZ%=0 THEN INPUT"DIRECTION <R/L>":RL$:ZZ%=1
1870IF RL$="R" OR RL$="L" THEN 1880 ELSE ZZ%=0:GOTO1860
1880REM DON'T CONTINUE UNTIL SPEAKER IS SPECIFIED
1890IF RL$="L" AND OUTFR>40 THENSOUND 1,-10,53,20:PRINT"EXCEEDING
FR. RANGE OF SPEAKER":T%=TIME:REPEATUNTIL TIME>T%+500:GOTO 2050
1900INPUT "ATTENUATION";ATTEN%
1910IF ATTEN%<=0 OR ATTEN%>100 THEN ARROW%=1:GOTO 2050
1920PROCspl
1930PROCpoints
1935REM STORE COORDS IN ARRAY
1940ACOUNT=ACOUNT+1
1950PTS(ACOUNT,1)=XPOS
1960PTS(ACOUNT,2)=YPOS
1970INPUT "NEXT FR?<RET>: JOIN POINTS?<1>: STORE DATA?<2>";OPT
1980IF OPT<1 OR OPT>2 THEN ARROW%=1:GOTO 2050
1990IF OPT=1 THEN PROCjoinup:GOTO 1970
2000IF OPT=2 THEN PROCstore:GOTO 1970
2010INPUT "OLD GRAPH OR NEW <DEF. OLD>? 1/2";IQ
2020IF IQ=2 THEN CLG:ZZ%=0:PROCaxes
2030ACOUNT=0
2040ARROW%=1
2050ENDPROC
2060
2070DEF PROCpoints
2080REM CALCULATE X AND Y COORDS IN GRAFIX UNITS
2090LGX=LOG(OUTFR)
2100XPOS=XMIN+(XSCL*LGX)
2110YPOS=YMIN+(SPL%-20)*(YMAX-YMIN)/80
2120PROCcross
2130ENDPROC
2140
2150DEF PROCjoinup
2160REM JOIN THE PTS WITH A LINE
2170PROCsort
2180FOR I=1 TO ACOUNT
2190IF I=1 THEN MOVE PTS(I,1),PTS(I,2) ELSE DRAW PTS(I,1),PTS(I,2)
2200NEXT
2210ZZ%=0
2220ENDPROC
2230
2240DEF PROCstore
2250REM STORE DATA TO DISC AS STRINGS
2260PROCsort
2270INPUT "FILE NAME ";FILE$
2272ERRCHK%=0
2274PROCfnchk(FILE$)
2276IF ERRCHK%<>0 THEN 2270
2280INPUT "IDENTIFICATION ?";IDENT$
2290X=OPENOUT FILE$
2300PRINT£X,IDENT$
2310PRINT£X,RL$
2320FOR N=1 TO ACOUNT
2330X$=STR$(PTS(N,1)):Y$=STR$(PTS(N,2))
2340PRINT£X,X$,Y$
```

```
2300PRINT£X,IDENT$
2310PRINT£X,RL$
2320FOR N=1 TO ACOUNT
2330X$=STR$(PTS(N,1)):Y$=STR$(PTS(N,2))
2340PRINT£X,X$,Y$
2350NEXT
2360CLOSE£X
2370REM CLEAR THE ARRAY FOR NEW DATA
2380FOR N%=1TO 40
2390PTS(N%,1)=0:PTS(N%,2)=0
2400NEXT
2410ZZ%=0
2420ENDPROC
2430
2440DEF PROCsort
2450REM RIPPLE SORT DATA INTO ASCENDING ORDER OF FR.
2460Z=0:N=0
2470N=N+1
2480IF PTS(N,1)>PTS(N+1,1) THEN PROCswap
2490IF Z=1 THEN 2460
2500IF N=ACOUNT-1 THEN GOTO 2520
2510GOTO 2470
2520ENDPROC
2530
2540DEF PROCswap
2550V=PTS(N,1)
2560V1=PTS(N,2)
2570PTS(N,1)=PTS(N+1,1)
2580PTS(N,2)=PTS(N+1,2)
2590PTS(N+1,1)=PTS(N,1)
2600PTS(N+1,2)=PTS(N,2)
2610PTS(N+1,1)=V
2620PTS(N+1,2)=V1
2630Z=1
2640ENDPROC
2650
2660DEF PROCcross
2665REM DRAW AN OPEN SQUARE (SIZE 2*NX)
2670MOVE XPOS+NX,YPOS+NX
2680DRAW XPOS-NX,YPOS+NX
2690DRAW XPOS-NX,YPOS-NX
2700DRAW XPOS+NX,YPOS-NX
2710DRAW XPOS+NX,YPOS+NX
2720MOVE XPOS,YPOS
2730ENDPROC
2740
2750DEF PROCspl
2755REM LOOK UP CORRECT ATTEN VALUE FROM CAL. CURVE
27600%=2
2770IF RL$="L" THEN 0%=3
2780XX%=0:YY%=0
2790FOR N%=1 TO 23
2800IF XX%<>0 THEN 2820
2810IF OUTFR<=DB(N%,1) THEN XX%=1:YY%=N%
2820NEXT
2830SPL%=DB(YY%,0%)-ATTEN%
2840ENDPROC
2850
2860REM READ A MEMORY ADDRESS
2870DEF FNREADIO(addr):!X%=addr: A%=5: CALL OSWORD
2880=X%?4
2890REM WRITE TO A MEMORY ADDRESS
2900DEF PROCwriteIO(data,addr):!X%=addr:X%?4=data
2910A%=6: CALL OSWORD: ENDPROC
2920
2930DEF PROCfnchk(AFILE$)
2940REM ENSURE THAT THE PROPOSED FILE NAME IS LEGAL
2950A=LEN(AFILE$)
2960IF A=0 OR A>7 THEN GOTO 3030
```

```
2930DEF PROCfnchk(AFILE$)
2940REM ENSURE THAT THE PROPOSED FILE NAME IS LEGAL
2950A=LEN(AFILE$)
2960IF A=0 OR A>7 THEN GOTO 3030
2970IF INSTR(AFILE$," ")<>0 THEN GOTO 3030
2980IF INSTR(AFILE$,"!")<>0 THEN GOTO 3030
2990IF INSTR(AFILE$,"*")<>0 THEN GOTO 3030
3000IF INSTR(AFILE$,":")<>0 THEN GOTO 3030
3010IF INSTR(AFILE$,".")<>0 THEN GOTO 3030
3020GOTO 3050
3030PRINT "INVALID FILE NAME":SOUND 1,-10,53,20:T%=TIME
3035REPEAT UNTIL TIME>T%+300
3040ERRCHK%=1
3050ENDPROC
```

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END