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Processing of Song Signals in the Cricket and its Hormonal Control[1]

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SYNOPSIS. Phonotaxis by female crickets to the calling song of males, is an important model for investigating the neural basis of auditory behavior. Recent advances make it possible to explain some components of this behavior and its hormonal control, at the level of identified neurons and molecular expression within those neurons.

Tonotopically arranged afferents from the cricket's ear, project to local and intersegmental prothoracic interneurons. Bilateral processing of signals and some temporal-pattern specific processing occurs in the prothoracic ganglion and influences acoustic information that is sent to the brain via ascending interneurons that are demonstrably involved in phonotaxis. High, low and band- pass interneurons in the brain continue temporal pattern processing which matches the selectivity of phonotaxis and may be filters for recognition of the calling song. Neurons descending from the brain and prothoracic ganglion, direct multimodal signals (including auditory) to more posterior regions, possibly the leg motor neurons that are responsible for phonotaxis.

Age-related changes or artificially induced changes in Juvenile Hormone III levels regulate the threshold for phonotaxis in *Acheta domesticus,* by varying the threshold of L1, a prothoracic ascending interneuron that is necessary for phonotaxis to low intensity calling songs. Results from *in situ* hybridization suggest that this might be accomplished, in part, by controlling the levels of nicotinic acetylcholine receptor-like mRNA expressed in L1, presumably by increasing its neurotransmitter receptor density. L3 is a prothoracic ascending interneuron that exhibits band-selective response properties to the syllable period of the calling song. L3's response is age and JHIII related, and is correlated to phonotactic selectivity. These changes in L3 might be accomplished, at least in part by JHIII regulating the expression of nicotinic acetylcholine receptor-like mRNA in L3.

INTRODUCTION

Within the field of neuroethology, several model systems have been investigated in an attempt to determine how biologically relevant auditory signals are recognized and localized and how such signals induce appropriate behavioral responses. In this paper, we will examine how the central neurons of the female cricket process information contained in the calling song of males which leads to phonotactic behavior by females.

Positive phonotaxis by the female cricket occurs in response to natural and model calling songs of the conspecific male (*e.g.,* Popov and Shuvalov, 1977; Thorson *et al.,* 1982; Schmitz *et al.,* 1982; Stout *et al.,* 1983; Stout and McGee, 1988). Selectivity of phonotactic orientation indicates that the nervous system must be able to distinguish between sounds on the basis of spectral and temporal features. In several species, the syllable period of the calling song is the most important temporal feature that determines the attractiveness of the call. If an attractive calling song is encountered, the female will

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walk towards the sound source. This indicates that the nervous system must be able to encode the direction of the sound source. In addition, the onset of, and control of walking must also be accomplished in response to appropriate sound signals. Behavioral experiments using stimuli patterns that arise from two sources (on their own neither one is attractive to *Gryllus* but combined they resemble attractive song patterns) demonstrate that localization of the sound source and recognition are complex and might be processed separately by different components of the central nervous system (Weber and Thorson, 1988; Doherty, 1991). In addition, manipulation of the temperature of the head and/or thorax of *G. firmus* during phonotaxis (Pires and Hoy, 1992) demonstrates that the process of recognition of the calling song is distributed within the cricket's nervous system. Modulation of phonotaxis by *Acheta domesticus* by Juvenile Hormone III (JHIII) indicates that components of the behaviors mentioned above must also be regulated (Walikonis *et al.*, 1991). That all of these processes can be evaluated at the identified cell level, and in some cases at the molecular level, makes this system an excellent model for studying the neural and hormonal control of behavior.

Several other aspects of audition in crickets have also been studied and reviewed, including mechanisms of the hearing organ (Nocke, 1972; Oldfield *et al.*, 1986; Boyan, 1993), territoriality and aggression in males (Nolen and Lam, 1990; Nolen *et al.*, 1991), and predatory avoidance (Moiseff *et al.*, 1978; Nolen and Hoy, 1986*a,b*; Hoy, 1992), and thus will not be addressed here. This paper highlights the processing of the male's calling song at the various neuronal levels of the female's auditory system thus enabling comparison with the processing of species-specific mating calls of frogs and grasshoppers (reviews in this issue). Important issues that still need to be resolved at each of these levels will be emphasized.

Figure 1 summarizes the major populations of identified neurons and pathways which respond to the calling song of males (carrier frequency of 4–5 kHz) and is intended to act as a guide through the various levels of the cricket's nervous system as each is addressed. Most of the connections between the groups of neurons have not been established, but are based on inferences from overlapping dendritic fields and axonal projections. In addition, the groupings are somewhat artificial in that some neurons within one group may have very different functions compared to others in the same group. Also, some neurons share characteristics of more than one group. For example, some prothoracic interneurons have both ascending and descending axons (T-neurons, Wohlers and Huber, 1985; Atkins and Pollack, 1987*a,b*) and thus share characteristics of both groups.

Local Prothoracic Neurons

Auditory sensory neurons are activated in the ear of the cricket which is located on the tibia of the prothoracic legs (Nocke, 1972; Oldfield *et al.*, 1986). These project to the anterior Ring Tract of the prothoracic ganglion where they activate ascending neurons (Hennig, 1988).

The omega shaped neuron, ON1 (Fig. 2A), occurs as a bilaterally symmetrical pair. Although these neurons are usually referred to as local interneurons, during development ON1 (and a similar pair of neurons, ON2—Wohlers and Huber, 1985; Stiedl *et al.*, 1993) has a functional ascending axon projecting towards the brain suggesting an interganglionic role (Atkins and Pollack, 1986; Fig. 2B). It is also interesting that the origin of this axon correlates well with the origin of some of the soma-contralateral sprouting that follows deafferentation experiments (Fig. 2C, Schildberger *et al.*, 1986).

The focus of experimentation of the response and function of the ON1 has been on its intraganglionic processing of sound signals. Using legphones (closed sound field for each ear with the tracheal interconnection between the ears removed), Kleindienst *et al.* (1981) showed in *G. campestris* that ON1 is excited by soma-ipsilateral sounds and is inhibited by tones from the contralateral side. When both ON1s are recorded intracellularly, a short constant latency IPSP is observed suggesting a direct mutually inhibitory connection between the pair of

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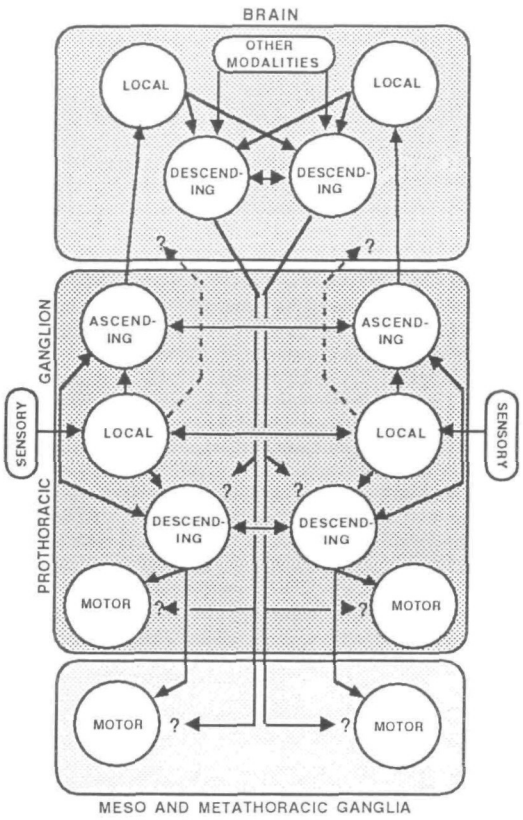

FIG. 1. Schematic diagram of the sites of auditory processing in the nervous system of the cricket. Arrows indicate overlapping dendritic regions within the major populations of auditory neurons that have been investigated in the brain and thoracic ganglia.

ON1s (Fig. 2D, Selverston *et al.*, 1985). Such contralateral inhibition has been suggested to be involved in enhancing directional information (Wohlers and Huber, 1982; Selverston *et al.*, 1985), temporal pattern filtering (Weise and Eilts-Grimm, 1985) and improving signal-to-noise ratio of the ascending neurons (Pollack, 1986, 1992; Sobel and Tank, 1994). These functions are only possible if the ON1s impinge on the responses of the ascending units. An inhibitory connection between ON1 and a contralateral ascending neuron has been demonstrated for at least the AN2 in *G. campestris* where photoinactivation of the ON1 while recording from AN2 resulted in a gradual loss of inhibition (Selverston *et al.*, 1985; Fig. 2E). Such inhibition would enhance the coding of directional information available to the brain via the ascending neurons by increasing the apparent intensity difference between the left and right ascending pathways. Electron microscopy and immunogold staining of ON1s that were labeled with intracellular injection of HRP, showed that ON1 does receive GABA-like inputs; however, ON1s are not immunoreactive for GABA (Hardt and Watson, 1993). This suggests that ON1s are not GABAergic and that they might inhibit each other via polysynaptic pathways. Even at this first level of central processing, it is becoming clear that simple networks involving ON1 may not adequately explain local neuronal processing (see also Atkins *et al.*, 1992).

In addition, electron microscopy has shown mixed input and output synapses on the soma-ipsilateral side of ON1 which indicates that local, possibly non- spiking interactions, are also involved. Spiking and non-spiking encoding in ON2 (Fig. 2F) and non-spiking activity in other, unidentified neurons may also play crucial roles in auditory processing. These possibilities need to be more fully evaluated in order to understand the roles of local prothoracic interneurons in phonotaxis to the calling song.

ASCENDING PROTHORACIC NEURONS

Intracellular recordings reveal the morphology and response properties of two (Wohlers and Huber, 1982, 1985; Hennig 1988) or three bilaterally symmetrical pairs of ascending neurons (Boyd *et al.*, 1984; Stout *et al.*, 1985, 1988; Atkins *et al.*, 1989*a,b*; Stumpner *et al.*, 1994) within the prothoracic ganglion. These neurons overlap with the terminals of the sensory neurons (Hennig, 1988) and the local prothoracic interneurons mentioned above, and they have an axon projecting to the brain on the side ipsilateral to their major inputs.

The behavioral roles of these neurons in the cricket have been clarified in *A. domesticus* by comparing phonotaxis before and after photoinactivation of one of the interneurons (Atkins *et al.*, 1984, 1992). Following a series of phonotaxis trials (usually a range of songs having different syllable periods and intensities) on a non-compensating treadmill (Walikonis *et al.*, 1991), one of

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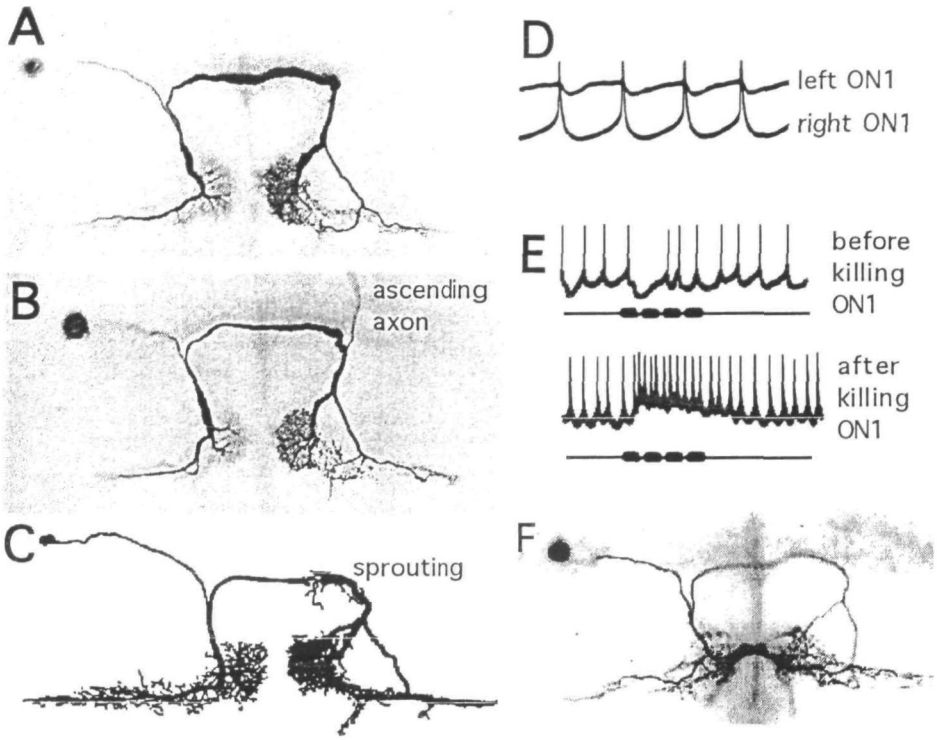

FIG. 2. Local prothoracic interneurons. **A-B.** Video images of wholemounts of ON1 filled with Lucifer Yellow from *T. oceanicus*. **A** without an ascending axon and **B** with an ascending axon (from Atkins and Pollack, 1986). **C.** Drawings of ON1 from *G. bimaculatus* following deafferentation on the soma-contralateral side. Note the sprouting which occurs at the same area where the ascending axon is found in young ON1s (taken from Schildberger *et al.*, 1986). **D.** Simultaneous recordings from the left and right ON1s in *G. bimaculatus* showing short constant latency PSPs in response to spiking in the other neuron (from Selverston *et al.*, 1985). **E.** Responses of AN2 before and after photoinactivating the contralateral ON1. Note the loss of inhibition in AN2 (from Selverston *et al.*, 1985). **F.** Video image of a wholemount of ON2 (stained with Lucifer Yellow) from *A. domesticus*.

the ascending neurons is penetrated with a microelectrode and Lucifer Yellow is iontophoretically injected into that neuron followed by rapidly killing the neuron using the light from a HeCd laser. In order to determine the neuron's role, phonotaxis posttests are then performed and compared to the results of the pretests. In some cases, the ear providing input to the intact side is occluded with wax to block activity in all neurons on that side and "one-ear" phonotaxis is examined (Kohne *et al.*, 1992; Atkins *et al.*, 1992).

In *A. domesticus*, two of the three ascending interneurons (L1, threshold = 50 dB, and L3, threshold = 70 dB) respond to 5 kHz calling songs. When L1 is inactivated, error angles in orientation away from the calling song source and circling toward the side providing input (axon ipsilateral) to the intact L1 (Fig. 3A) occur when the intensity of the song is 60 dB (a situation where only one L1 would be responding). Error angles are greatest in response to ideal syllable periods (50–60 ms, Fig. 3B). Syllable period-dependent circling exists with most circling occurring in response to the ideal syllable periods (Fig. 3C), indicating that at these intensities, activity in one L1 is sufficient for recognition of the calling song. When the ear providing input to the intact side is blocked, L1, L2 and L3 are not active and no phonotaxis occurs thus demonstrating L1's necessity (Fig. 3D).

When an L3 is inactivated, angular errors occur when phonotaxis is tested using inten-

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sities that are above the threshold of L3 (Fig. 3E). This shows that L3 is involved in phonotaxis. The smaller angular errors compared to those when L1 is inactivated, might suggest a less important role for L3. However this may also be due to the activity in the matched pair of L1s which would help offset the effects of inactivating one L3. At lower intensities, no consistent errors are noted and therefore acts as a control for the photoinactivation procedure (Atkins *et al.*, 1992). Additional support for the function of L3 comes from experiments where one L1 is inactivated and the ear providing input to the intact side is occluded with wax (Fig. 3F) which results in only one L3 functioning of the six ascending neurons (at 85 dB). Activity in a single L3 leads to phonotactic circling and large error angles, thus strengthening the argument that L3 might be involved in localization and recognition. When the sound intensity is dropped below the intact L3's threshold, orientation ceases (Atkins *et al.*, 1992). The involvement of L1 and L3 in localization described here is consistent with experiments with *Gryllus* where the roles of two ascending neurons (AN1 and AN2—both responding to 5 kHz songs) were evaluated by hyperpolarizing one of the neurons during walking phonotaxis (Schildberger and Horner, 1988). They found that AN1 and AN2 were involved in localization during phonotaxis; however their procedure did not evaluate the neurons' possible roles in recognition.

While photoinactivation experiments reveal the roles of L1 and L3, little is known about the circuits that underlie the response properties of these neurons or the nature of potential outputs to their bilateral partners or other prothoracic interneurons. Circuit analysis and continued ultrastructural studies like those which have been accomplished with ON1 (see above) are needed to clarify how these neurons which are involved in phonotaxis, obtain the appropriate signals and how their spiking and/or non-spiking signals influence post-synaptic neurons.

HORMONAL CONTROL OF PROTHORACIC NEURONS

JHIII is a common insect hormone that has been shown to effect major changes in insect nervous systems including the differentiation of brain neurons in adult *A. domesticus* (Cayre *et al.*, 1994). Although produced by the corpora allata and not necessary for phonotaxis under certain conditions (Loher *et al.*, 1992), JHIII clearly influences several aspects of phonotaxis by female *A. domesticus*, including the threshold of the response and selectivity for the syllable period of the calling song (Walikonis *et al.*, 1991; Stout *et al.*, 1991). The effect of JHIII is illustrated in two prothoracic interneurons where it, at least in part, increases their excitatory input (Stout *et al.*, 1991; Henley *et al.*, 1992). Support for the hypothesis that this increased excitability results from JHIII increasing the expression of the locust ACh receptor-like mRNA in these neurons, comes from behavioral, local hormonal applications (Stout *et al.*, 1991), and double-labelling *in situ* hybridization experiments, the results of which will be summarized here (see also Stout *et al.*, 1992, 1994).

Following behavioral tests, auditory interneurons are recorded from intracellularly and stained with Lucifer Yellow. Prothoracic ganglia containing these stained neurons are excised, fixed, sectioned, identified and photographed. *In situ* hybridization is performed using oligodeoxyribonucleotide probes derived from the nucleotide sequence of the α-L1 nicotinic ACh receptor gene (Marshall *et al.*, 1990) of the Locust (*Shistocerca gregaria*) which are conjugated to bovine alkaline phosphatase as a histological marker. A cell is considered hybridized when dense, dark labeling, is found within the cytoplasm of the soma and when visible, delineates a clear unlabeled nucleus (Baldino and Lewis, 1989; Stout *et al.*, 1992; Emson, 1993). Hybridized somata are evaluated densitometrically using image processing software (NIH Image).

Threshold for phonotaxis in female *A. domesticus* drops between days 2–4 following the imaginal molt and is correlated with increasing rates of JHIII synthesis by the corpora allata (Walikonis *et al.*, 1991). The spiking threshold of the L1 auditory interneurons (described above as necessary for phonotaxis at low intensities) also change in a correlated manner with thresholds

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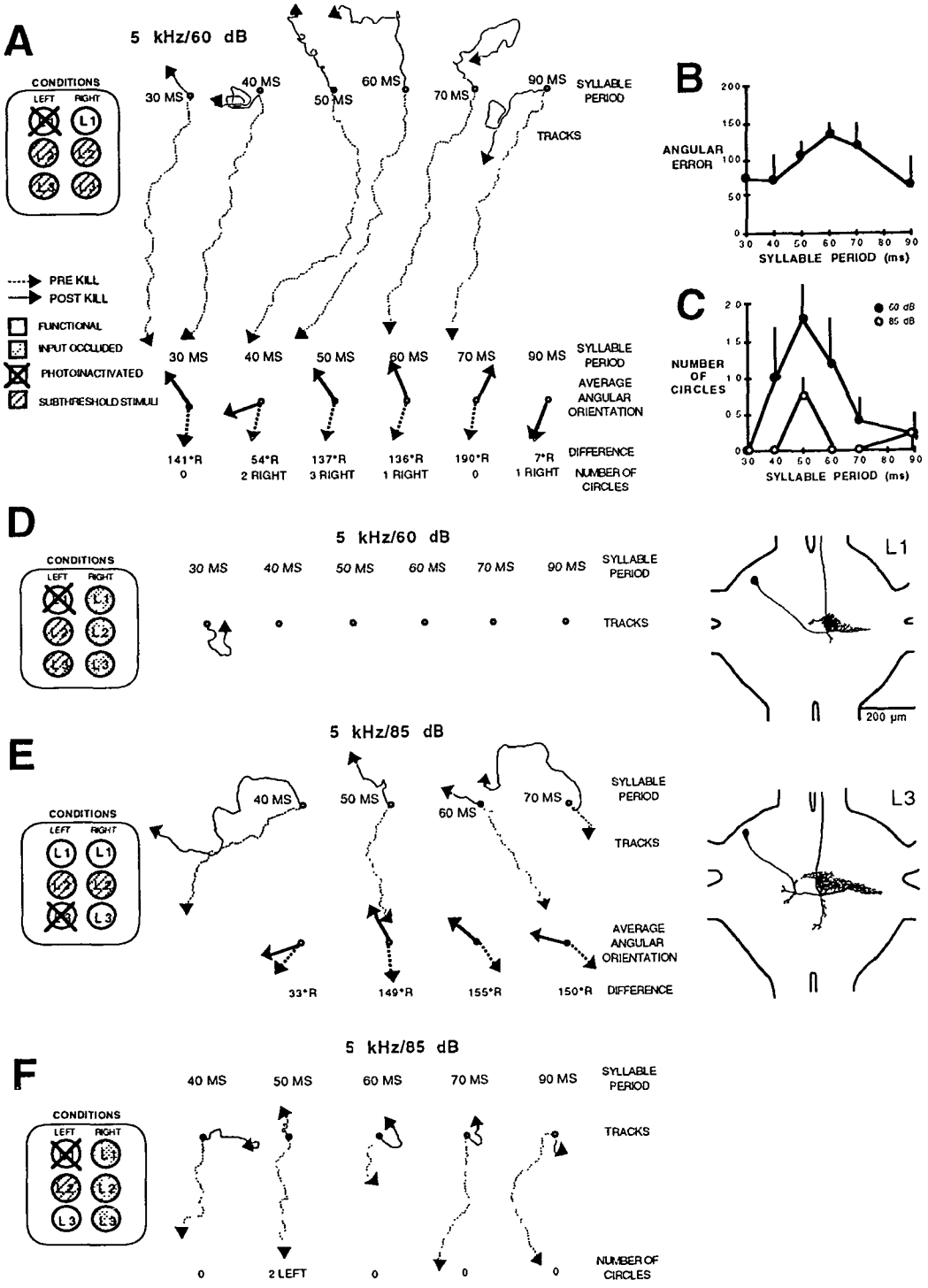

FIG. 3. Roles of ascending prothoracic interneurons in *A. domesticus*. **A.** Effects of killing one L1. Tracks and average orientation angles for a female in response to calling songs having various syllable periods at 60 dB. The speaker position would be below the figure in each case. **B.** Average angular errors following inactivation of one L1 as in **A** (resulting from turning toward the side providing input to the intact L1) for 5 females. **C.**

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dropping from 80dB to 50dB by day 4 (Fig. 4A). The somata of L1s show little expression of the nicotinic receptor-like mRNA in 1-day-olds (Fig. 4B) but show strong expression in 4-day-olds (Fig. 4C). This suggests that JHIII might regulate phonotactic threshold by regulating the sensitivity of L1 to sensory neuron input.

In female *A. domesticus,* the most important temporal parameter of the calling song for phonotaxis is the syllable period (Stout *et al.,* 1983; Stout and McGhee, 1988). Young females (4–7 days) respond selectively to calling songs with syllable periods of 50– 70 ms being most attractive. As females become older they are less selective for syllable period (ie. respond to a wider range of syllable periods). By 14 days following the imaginal molt, females are not selective and respond to calling song syllable periods ranging from 25–100 ms (Walikonis *et al.,* 1991). L3 is an ascending prothoracic interneuron which is involved in phonotaxis (see above), and responds in a band-selective manner to syllable period (Fig. 4D). In young females, L3 decrements maximally (decrease in the number of action potentials produced in response to the third syllable of the call compared to that of the first) in response to calling songs having syllable periods of 50–70 ms (the range of the conspecific calling song), with less decrement in response to those having longer and shorter syllable periods (Fig. 4 D, Atkins *et al.,* 1989*b*). Band-selective decrement might be important for improving the signal to noise ratio of the encoding of the syllables of the ideal calls. Thus less attractive calls would not be encoded as well as would the conspecific call (Atkins *et al.,* 1989*b*). L3s response represents temporal pattern processing at the level of the prothoracic ganglion and may be a neural correlate for the selective heating experiments by Pires and Hoy (1992) which showed that both the thorax and the head were necessary for temporal pattern recognition. L3s from young females that display syllable period-selective phonotaxis, show clear expression of nicotinic receptor-like mRNA (Fig. 4E). The response and expression in L3 changes with age. In older females (28 days), L3 produces fewer action potentials in its response (particularly in response to the first syllable period of the chirp) and is not selective for syllable period (*i.e.,* does not exhibit band-selective decrement). These L3s express little nicotinic receptor-like mRNA (Fig. 4F). Topical application of JHIII to the abdomen of old females, which have previously responded to songs having syllable periods ranging from 30–90 ms before hormone application, only respond well to calling song syllable periods of 50–70 ms following JHIII application. L3's response correlates with this effect on behavioral selectivity. JHIII application to old animals (normally unselective) results in clear decrement of response in L3 and greater expression of nicotinic receptor-like mRNA than do untreated females (Fig. 4G).

The regulation of the threshold and selectivity of L1 and L3, at least partially illustrates the mechanism of hormonal control of behavior and could provide a model system where the regulation of a complete, rather complex and essential behavioral response by female *A. domesticus* is accomplished by JHIII's regulation of the response properties of identified auditory neurons. Although it cannot be considered demonstrated that JHIII controls phonotaxis by regulating the concentration of acetylcholine-like receptors, the changes in mRNA expression in L1 and L3 suggest that it is the most consistent interpretation of the mechanism of control by JHIII. Several issues must be considered however, before more direct conclusions can be made. 1) The oligodeoxynucleotide probes used in these studies are based on conserved regions of the Locust α-L1 nicotinic acetylcholine-

←

orientation trials. Error bars represent standard error of the mean. D. Effect of occluding one ear and killing one L1. Tracks of a female in response to calling songs having various syllable periods at 60 dB (below L3's threshold). Inset shows typically morphology of L1. E. Effect of killing one L3. Tracks and orientation angles for a female in response to various syllables at 85 dB. Inset shows typical morphology of L3. F. Tracks and circling of one female following occlusion of one ear and killing one L1—leaving one L3 functioning (from Atkins *et al.,* 1992).

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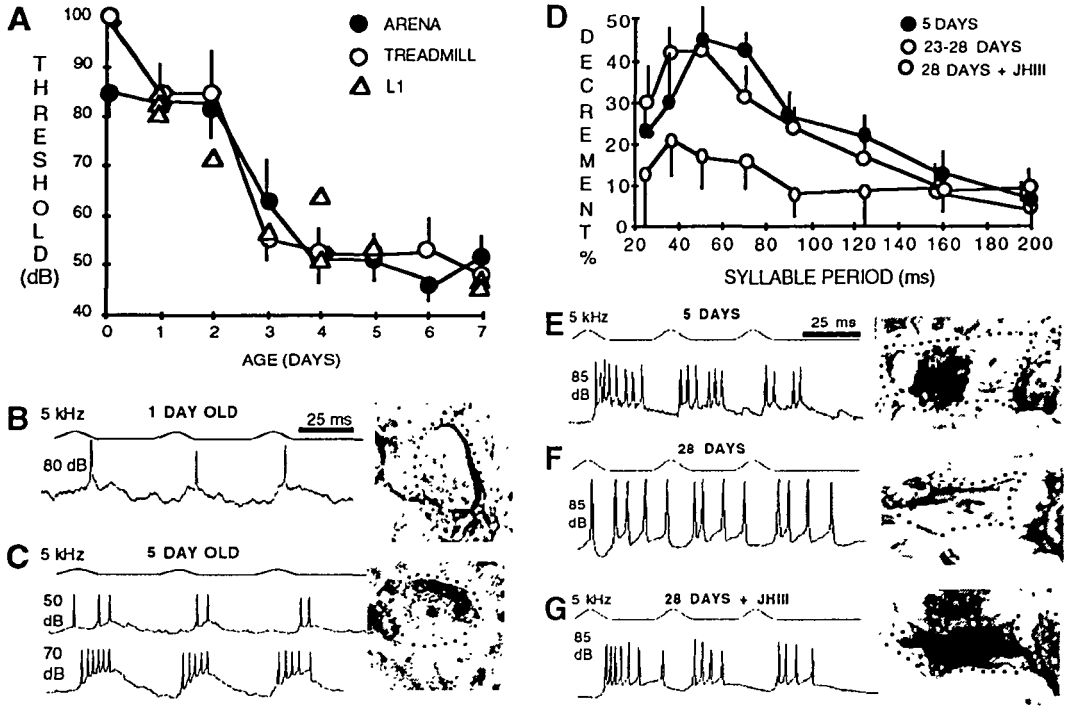

FIG. 4. JHIII influences behavior through molecular changes in prothoracic interneurons. **A.** Graph showing the age- related changes in phonotactic threshold (tested in a circular arena and on a spherical, noncompensating treadmill) and the thresholds of the L1 auditory interneuron in adult *A. domesticus* (from Stout *et al.*, 1991). **B-C.** Sample responses (to a three syllable chirp shown above each trace) and 20 μm sections containing the somata (outlined, based on double labeling with Lucifer Yellow) following *in situ* hybridizations probing for α-LI nicotinic acetylcholine receptor-like mRNA for L1s in **B**, a one-day-old, and **C**, a 5 day-old. **D.** Graph showing the effect of age and JHIII application on syllable period-dependent decrement in L3. Note that 5 day-olds and JHIII- treated 28 day-olds show the greatest decrement in response to syllable periods around 50 ms (the modal value of the conspecific calling song, modified from Henley *et al.*, 1992). **E-G.** Sample responses and sections containing the somata following *in situ* hybridizations probing for α-LI nicotinic acetylcholine receptor-like mRNA for L3s in **E**, a 5 day-old, **F**, a 28 day-old, and **G**, a 28 day-old following application of JHIII. In each case, the responses and sections are taken from the same preparation (modified from Stout *et al.*, 1994).

like receptor gene and thus might be probing a family of genes and not specifically the cricket α-L1 nicotinic acetylcholine receptor. 2) *in situ* hybridization identifies expression of mRNA and not the presence or amount of protein present. Other histological techniques need to be applied (Hockfield *et al.*, 1993) in order to demonstrate the existence of the receptor protein and it's concentrations relative to JHIII levels and age.

Whether JHIII receptor-like proteins are present in these neurons, or if JHIII has its effects indirectly on these interneurons must also be determined. Further, it has become clear that factors other than age-dependent regulation by JHIII may be influencing this system. The density of females during nymphal development as well as different experimental techniques seem to influence the detection of the time course of phonotactic threshold changes (Atkins *et al.*, 1993). Also, there seems to be short-term (several hours) and long- term (several days) effects of JHIII levels on selectivity to syllable period (Burghardt *et al.*, in preparation).

The changes in the excitability of L1 and L3 could also be influenced by JHIII regulating inhibitory transmitter receptors. IPSPs do exist in these neurons in *Acheta,* and since recent ultrastructural and immunological studies have shown GABA inputs onto these neurons in *Gryllus* (Hardt and Watson, 1993), *in situ* hybridization must

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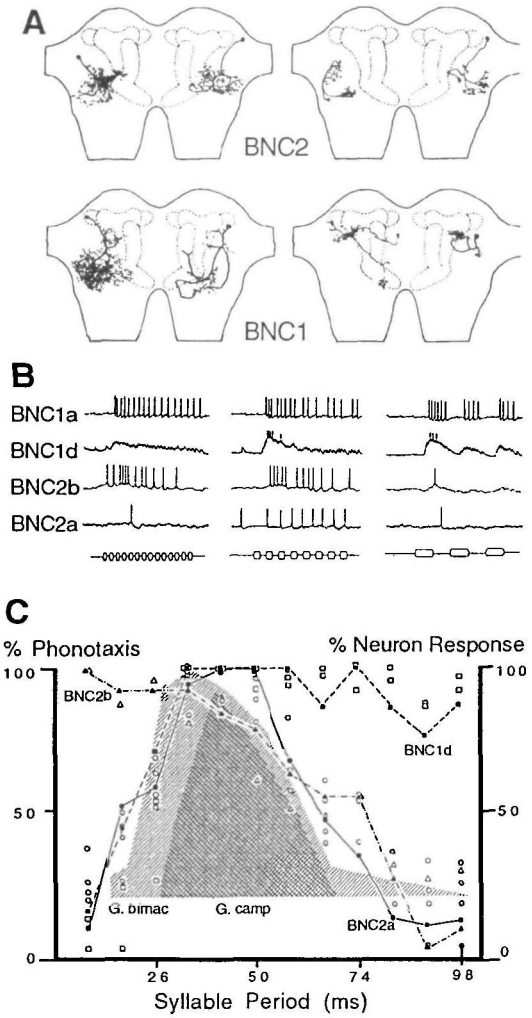

FIG. 5. Temporal filtering in local brain interneurons in *G. campestris*. A. Drawings of wholemounts of BNC1 and BNC2 neurons in the brain. B. Sample responses of some BNC1 and BNC2 neurons in response to different temporal patterns. Note that some neurons show low, high or band-pass filtering properties. C. Graph showing the responsiveness of filtering neurons of *G. campestris* (lines) and the phonotactic responsiveness (shaded areas) of two *Gryllus* species to the same model calls having different syllable periods (modified from Schildberger, 1988).

be continued for GABA receptor-like mRNA; the nucleotide sequence for an insect GABA receptor has just been determined (Henderson *et al.*, 1993). Furthermore we cannot rule out the possibility of second messenger-type responses to JHIII. Thus, the simplistic models described above must not yet rule out other possible mechanisms of regulation.

LOCAL BRAIN NEURONS

Ascending neurons from the prothoracic ganglion project primarily to a ring-shaped neuropil region in the protocerebrum of the supraesophageal ganglion. Here, they overlap with BNC1 neurons which are local neurons that overlap with more posteriorly positioned BNC2 neurons (Fig. 5a, Schildberger, 1984, 1988). Evaluation of these neurons is based on their spiking patterns in response to various models of the calling song in comparison to the behavioral response of females when these songs are used in phonotaxis tests.

While some local brain neurons respond to all songs tested (BNC1a, Fig.4B), some act as "high-pass" neurons; responding with action potentials when the pulse frequency is above a certain value (BNC2b, Fig. 5B). Others respond as "low-pass" filters; action potentials are produced in response to lower pulse frequencies (BNC1d, Fig. 5B). These filtering properties are thought to be a function of the synaptic properties of the brain neurons themselves rather than filtering that is occurring elsewhere being imposed on the brain neurons (*i.e.*, ascending neurons—one of which has been described in *A. domesticus* is band-selective [Atkins *et al.*, 1989*b*]). A few of the neurons display more complicated spiking patterns and act as "band-pass" neurons, responding only to song patterns within a certain range of pulse repetition rates (centered around 30 Hz for *G. campestris*, BNC2a, Fig. 5B). This property is consistent with low-pass and high-pass neurons being integrated onto a postsynaptic cell whose threshold is set so that input from both low and high pass neurons would summate above that threshold. Thus, this neuron would only respond to the range of stimulus patterns that are overlapped by the low and high-pass neurons involved (Schildberger, 1988). These filtering properties are similar to some of the units described at several levels in the anuran auditory system, although anurans display even more complex filtering properties (such as band-suppressive neurons; see the review by Hall, 1994) than do crickets.

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Many correlations exist between the filtering properties of the BNC units and phonotaxis. The cut-off of low-pass neurons parallels the decline in attractiveness of syllable period during phonotaxis (Fig. 5C). The cut-off of high-pass neurons parallel the decline in phonotaxis to longer pulse periods, and band-pass neurons correlate well with the overall behavioral tuning of phonotaxis (Fig. 5C). Although the original comparisons between the filtering characteristics of BNC neurons of *G. campestris* was done with the behavior from *G. bimaculatus* (Schildberger, 1984), more recent comparisons illustrate the correlations between the filtering properties of the brain neurons and the behavior of *G. campestris* (Schildberger, 1988; Fig. 5C). Thus the filtering properties of the band-pass neurons match the behavior ranges of both species and can not clearly distinguish between these two calls. This is reflected in the behavioral response of *G. campestris* where differentiation of the two calling songs is difficult (Thorson *et al.*, 1982).

Although good correlations exist between the neurons and behavior, experiments are needed, using photoinactivation (described above) or hyperpolarization of neurons during phonotaxis (described below), to determine the roles of the neurons described in the brain (many more auditory neurons exist in the brain than have been described in the prothoracic ganglion). In addition, circuit analysis similar to that described for local prothoracic neurons is needed to test if the hypothesized filtering properties of the BNC neurons are acquired from unfiltered inputs of the prothoracic ascenders or if temporal pattern processing in the prothorax (Atkins *et al.*, 1989*b*; Pires and Hoy, 1992) is necessary for filtering in BNCs.

Another interesting finding is that responses of BNC2 cells are essentially intensity independent (Schildberger, 1984). Thus, these neurons could not be involved in localization of the sound source although the responses of some are consistent with them being considered "recognizers" of the calling song. Sufficient direction-sensitive neurons have not been described at this level or in the descending brain neurons that initiate walking and orientation (see below). Thus, integration of directional information in the brain from the ascenders needs to be evaluated, along with those experiments suggested above, in order to clarify how the brain initiates and controls recognition and localization during phonotaxis.

DESCENDING BRAIN NEURONS

The role of identified brain neurons which descend to lower ganglia has been investigated by recording from neurons while the walking behavior of *G. bimaculatus* was monitored simultaneously. This approach has been used in investigating prothoracic interneurons used in flight phonotaxis (Nolen and Hoy, 1984) and in walking phonotaxis (Schildberger and Horner, 1988). Walking movements, which result in rotation of a light styrofoam ball that is in contact with the cricket's tarsi, is monitored electronically. Translational and rotational velocities which represent potential movement of the cricket could be measured during orientation while recording was done intracellularly and the desired interneuron stained. In addition, various patterns of stimuli could be presented (including non-auditory) and/or depolarizing or hyperpolarizing current could be injected while walking behavior was being monitored simultaneously. The result is that not only are neurons identified by their response properties to various stimuli, but their activity relative to behavioral output and the necessity of neurons for various portions of the orientation behavior can be clarified.

Bohm and Schildberger (1992) described at least five descending neurons, many of which are multimodal and some of which seem to be correlated with aspects of phonotaxis. Two will be discussed here. One of the Ipsilateral Descending Interneurons (IDIN, Fig. 6A) is only slightly responsive to auditory stimuli when the female is standing, but its sensitivity to sound increases dramatically when walking occurs (Fig. 6B). This is similar to some descending units during flight phonotaxis in crickets and during visually-guided steering in locusts (Tomioka and Yamaguchi, 1984; Rowell, 1988) where the responsiveness of the neurons depend on the behavioral context of the organism. Further, walking is positively correlated with spiking frequency of this interneuron. This suggests that it is respon-

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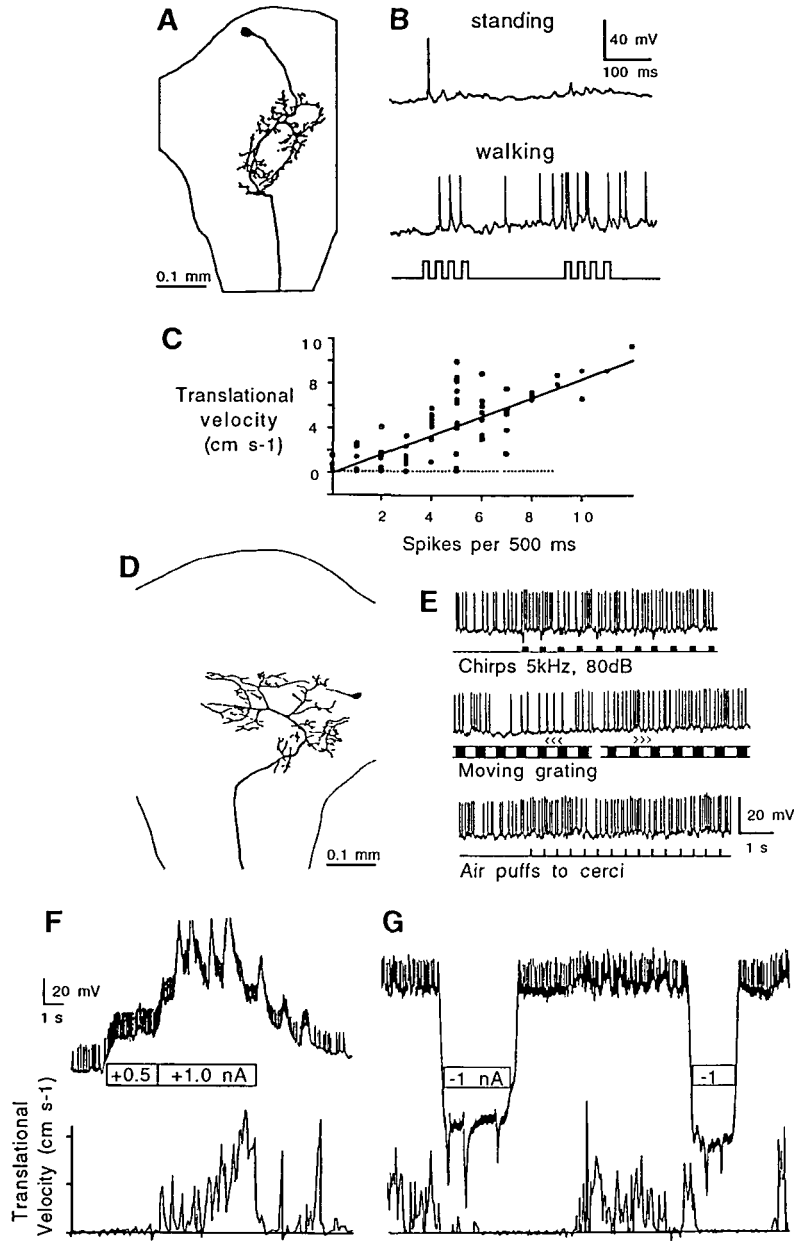

FIG. 6. Roles of descending brain interneurons in *G. bimaculatus*. **A.** Wholemount of a descending brain neuron. **B.** Comparison of the neuronal response to calling songs during walking and while standing. Sample recording (top) and histogram (bottom) for 16 successive chirps. **C.** Graph showing the correlation between the firing rate of the neuron shown in **A** and the simultaneously recorded walking velocity for 500 ms samples (n=40). **D.** Wholemount of a second descending brain neuron. **E.** Sample responses of the neuron shown in **D** to various stimuli. **F.** Activation of the neuron (top trace) and of walking (represented by translational velocity, bottom trace) by positive current injection. **G.** Deactivation of the neuron (top trace) and of spontaneous bouts of walking (represented by translational velocity, bottom trace) by negative current injection (modified from Bohm and Schildberger, 1992).

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sible for initiation of locomotion which might lead to phonotaxis (Fig. 6C). It is also possible however, that this neuron is responding to walking behavior itself, and that this response summates with auditory stimuli leading to increased spiking. The demonstration of the necessity of another multimodal IDIN neuron (Fig. 6D,E) was clearly shown by depolarizing the neuron with current injection while recording intracellularly in the absence of other stimuli. Strong enough depolarization initiates walking (Fig. 6F), whereas hyperpolarization can stop bouts of spontaneous walking (Fig. 6G). These data suggest that this multimodal interneuron is responsible, at least in part, for initiating walking that could lead to taxis in response to several stimuli including sound.

Data are not yet available which describe how directionality might be accomplished by the activity of descending brain interneurons during orientation. While it is possible that descending brain neurons might receive input from local brain neurons and prothoracic ascending neurons, none of the descending projections of these neurons have been shown to overlap with motor neurons that are responsible for phonotaxis nor has it been shown if other levels of processing are required. Further, the auditory responsiveness of many of these descending neurons is weak, often decrementing, and not as tuned to temporal patterns of the calling songs as are some of the local brain neurons mentioned earlier and thus seems less likely to play a major role in phonotaxis to calling songs. While Bohm and Schildberger (1992) have provided an important first analysis of the roles of identified descending brain neurons, clearly other descending neurons (see Boyan and Williams, 1981; Tomioka and Yamaguchi, 1984) must be employed to deliver direction-sensitive and temporal pattern selective information to motor areas involved in phonotaxis.

DESCENDING PROTHORACIC NEURONS

Several descending interneurons have been described in the prothoracic ganglion of *T. oceanicus* (Atkins and Pollack, 1987*a,b*). Some of these have dendritic fields in the auditory neuropil that overlap with sensory neurons and first-order auditory interneurons and may receive their input from sensory cells. However, many of these do not have such processes and therefore must rely on other inputs either from local prothoracic interneurons or from interneurons descending from the brain. The long latencies of some of these units (up to 58 ms, Atkins and Pollack, 1987*a*) and their multimodal responsiveness are both consistent with them receiving input from descending neurons in the brain such as those described above. At least some of these neurons overlap with the motor neurons and appear to make monosynaptic connections to motorneurons in mesothoracic ganglia and possibly metathoracic and lower ganglia (Fig. 7A). Since the threshold for some of these neurons is rather high it is possible that they do not serve important functions in phonotaxis. However, since the behavioral context is known to influence the responsiveness of some neurons (Tomioka and Yamaguchi, 1984; Nolen and Hoy, 1984; Bohm and Schildberger, 1992; Fig. 6C), the role of these neurons during phonotaxis needs to be determined (possibly by using techniques similar to those described above for descending brain neurons, or photoinactivated as described for the ascending neurons).

An additional feature of these prothoracic descending neurons is a crude tonotopic organization of the cells (Atkins and Pollack, 1987*b*). Low frequency tuned neurons are primarily located ventrally and ultrasound-tuned neurons are dorsally positioned within the prothoracic ganglion (Fig. 7B). Tonotopy is evident in the crista acoustica of crickets (Oldfield *et al.*, 1986), in the auditory neuropil of Tettigonidae (Oldfield, 1982) and in the CNS of anurans (Feng *et al.*, 1990; Hall, 1994). Tonotopy does not seem to be as pronounced in the CNS of crickets although physiological evidence exists for selective outputs of high and low frequency sensory neurons onto first order interneurons in crickets (Pollack, 1992).

Little information exists regarding which motor neurons are responsible for phonotactic orientation during walking. Clearly, descending information from the brain and prothoracic neurons is available and might

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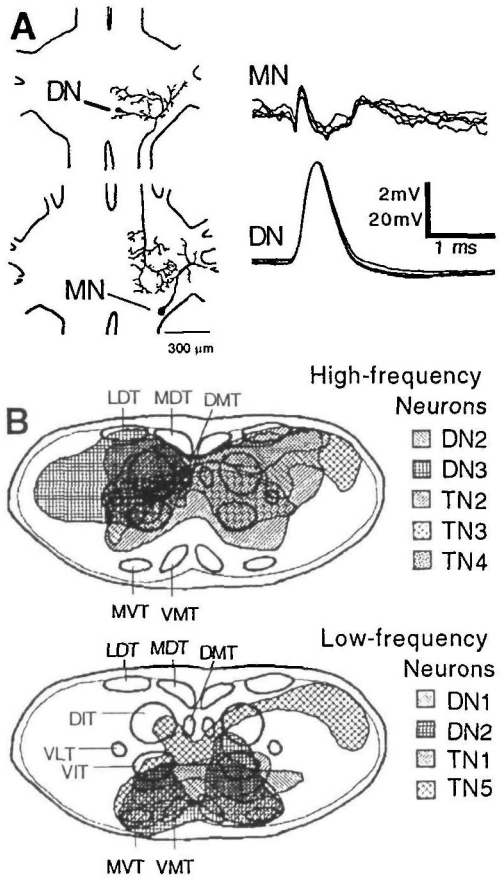

FIG. 7. Descending neurons in the prothoracic ganglion. **A.** Wholemount of a prothoracic descending interneuron with terminals overlapping the dendrites of a wing motor neuron in the mesothoracic ganglion that is involved in phonotaxis in *T. oceanicus*. Simultaneous recordings from the interneuron and motor neuron show a short constant latency EPSP (5 superimposed traces are shown). **B.** Topographic relationships of the neuropil segments of low and high-frequency descending neurons in the prothoracic ganglion of *T. oceanicus* (transverse plane, dorsal side up). Outlined areas do not include the soma and branchless portion of the neurite (from Atkins and Pollack, 1987*b*).

exhibit effects directly on the motor neurons. However no data exist to determine if local circuits exist between the descending neurons and the motor neurons in a manner similar to those described in the descending visual pathway in the locust (Rowell, 1988).

SUMMARY

Processing of the male's calling song by the female cricket occurs at many levels in the central nervous system. Several components and putative components of the processes of sound localization, call recognition, response initiation and hormonal control of behavior have been identified and evaluated at the cellular and for some, at the molecular level. While the mechanism for positive phonotaxis in response to the calling song has not yet been completely described, the ongoing successes in developing techniques to approach the cricket's auditory system give us encouragement that continued effort and innovation will bring us closer to an understanding of the cellular and molecular basis of phonotaxis. In addition, this effort and similar research with other model systems (described in this volume) will lead to a better understanding of the processes underlying animal communication in general.

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