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ORIGINAL PAPER

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Effects of maturation on synaptic potentials in the locust flight system

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Abstract We have measured parameters of identified excitatory postsynaptic potentials from flight interneurons in immature and mature adult locusts (*Locusta migratoria*) to determine whether parameters change during imaginal maturation. The presynaptic cell was the forewing stretch receptor. The postsynaptic cells were flight interneurons that were filled with Lucifer Yellow and identified by their morphology. Excitatory postsynaptic potentials from different postsynaptic cells had characteristic amplitudes. The amplitude, time to peak, duration at half amplitude and the area above the baseline of excitatory postsynaptic potentials did not change with maturation. The latency from action potentials in the forewing stretch receptor to onset of excitatory postsynaptic potentials decreased significantly with maturation. We suggest this was due to an increase in conduction velocity of the forewing stretch receptor. We also measured morphological parameters of the postsynaptic cells and found that they increased in size with maturation. Growth of the postsynaptic cell should cause excitatory postsynaptic potential amplitude to decrease as a result of a decrease in input resistance, however, this was not the case. Excitatory postsynaptic potentials in immature locusts depress more than in mature locusts at high frequencies of presynaptic action potentials. This difference in frequency sensitivity of the immature excitatory postsynaptic potentials may account in part for maturation of the locust flight rhythm generator.

Key words Insect · Motor pattern · Development · EPSP Synaptic depression

Abbreviations EPSP excitatory postsynaptic potential
fSR forewing stretch receptor
IPSP inhibitory postsynaptic potential
SR stretch receptor

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Introduction

Neuronal circuitry that generates motor patterns underlying behaviour is impressively plastic. It has been demonstrated that neural circuits can be reconfigured in the short term, by the presence or absence of particular neuromodulators, to produce a repertoire of different behaviours (e.g. the crustacean stomatogastric system, Harris-Warrick and Marder 1991). In a longer term developmental context circuitry can be modified to generate age-specific behaviours (e.g. Bekoff et al. 1987; Weeks et al. 1989). Not surprisingly, this ability of neural circuits to control different behaviours has generated considerable interest in the mechanisms underlying such circuit reconfiguration. Whereas information is accumulating on the short term plastic (McNaughton 1993) and elastic changes (Harris-Warrick and Marder 1991), there is less information available about what occurs to neuronal circuits as they are implemented at different developmental stages.

Post-embryonic plasticity in vertebrates has been demonstrated in the central auditory system in gerbils (Sanes 1993), seasonal plasticity of the birdsong nucleus (Nottebohm 1981) and the changes in synaptic efficacy and anatomy which are thought to underlie learning and memory (Lisman and Harris 1993). In these systems, however, it is difficult to relate changes in synaptic interactions of the circuitry to subtle changes in behaviour. In the more accessible systems of invertebrates it is possible to make such correlations. For example neurons, associated with proleg movements in *Manduca sexta* larvae, undergo regression of their dendritic arbors and depression of the strength of synaptic connections during pupation (Weeks et al. 1989). Dendritic reorganization of motoneurons during metamorphosis has been correlated with changes in their synaptic inputs (Levine and Truman 1982) and in the behaviour which they mediate (Jacobs and Weeks 1990; Kent and Levine 1993). In these studies, however, the changes in properties of the circuitry are accompanied by extensive changes in external morphology and the underlying musculature. In hemime-

tabolous insects, such as the locust, development proceeds along a more linear course without such drastic changes in external morphology and, in particular, of the musculature.

The flight circuit of *Locusta migratoria* is a useful system for examining what happens as a circuit for generating rhythmic behaviours first becomes functional. The central component of the flight motor pattern generator is present in all pre-adult stages but is not used until after the wings have emerged during the imaginal moult (Stevenson and Kutsch 1986, 1988). The output frequency of the flight motor, as indicated by the wingbeat, increases over the next 14 days (Kutsch 1974; Gray and Robertson 1994). This is a subtle modification in behaviour which is amenable to investigation at the neuronal level. The flight rhythm is generated at the interneuronal level (Robertson and Pearson 1984) but is modified by afferent input (Wolf and Pearson 1987). The change in output frequency of the flight motor pattern during maturation can be attributed largely to changes in proprioceptive input (Stevenson and Kutsch 1988). However, there may also be concurrent changes in flight interneurons that would affect their response to the afferent signal. Indeed there is evidence that the central circuit is changing because, following functional deafferentation, the output frequency of the central flight rhythm generator is greater in mature (>14 days past the imaginal moult) adult locusts than in immature (<2 days past the imaginal moult) adult locusts (Gray and Robertson 1994).

We set out to determine whether, during post-imaginal maturation of the flight motor output, there are changes in synapses from the forewing stretch receptor (fSR) to flight interneurons concurrent with increasing fSR sensitivity. There are 4 SRs, each is a single cell which is associated with a strand of connective tissue, one at the base of each wing (Gettrup 1962, Pabst 1965). The fSR branches extensively in the 3 thoracic ganglia (Altman and Tyrer 1977) and makes monosynaptic connections with many motoneurons (Burrows 1975) and interneurons of the locust flight circuit (Reye and Pearson 1987). The firing frequency of the SR increases with increasing wing elevation (Pabst 1965) and it has been shown that the SR can entrain the frequency of the central motor pattern (Reye and Pearson 1988). Thus the fSR is an important component of the locust flight system. By using action potentials in the fSR as the stimulus, and filling postsynaptic interneurons in the mesothoracic ganglion with Lucifer Yellow, we were able to compare parameters of EPSPs from identified synapses in immature and mature adult locusts.

Materials and methods

Male adult *Locusta migratoria* were obtained from a crowded colony which is maintained at the Department of Biology, Queen's University (31°C, 18:6 light:dark). Immature animals moulted two days or less prior to use. Mature animals were at least 13 days past the imaginal moult.

Preparation

Animals were cold-anaesthetized, the legs and wings were removed and a dorsal midline incision was made. The thoracic ganglia were then exposed and a stainless steel plate was placed beneath them to provide stability (Robertson and Pearson 1982). The body cavity was filled with saline at room temperature [in m*M*: 147 NaCl, 10 KCl, 4 $CaCl_2$, 3 NaOH, 10 HEPES buffer]. A silver wire hook electrode was placed on mesothoracic nerve $1D/1D_2$ (nomenclature of Campbell 1961) to record and/or stimulate the forewing stretch receptor (fSR) (Fig. 1). Nerves 3 and 4 of both meso- and metathoracic ganglia were cut to increase stability of the preparation. In some preparations, all thoracic nerves were cut or crushed and the pro-meso and abdominal connectives were crushed to reduce background synaptic activity. In later experiments, nerve $1D_2$ was cut distal to the hook electrode to eliminate spontaneous fSR activity and allow us to control the fSR action potential frequency by stimulating electrically. The stimuli were of short duration (0.2 ms) and suprathreshold (~12 V) to ensure that action potentials were generated rapidly. Using this stimulus regime the PSPs recorded from the interneurons were identical to those produced by spontaneous fSR action potentials. The PSPs were always unitary indicating that other afferents were either not being stimulated and/or they were not affecting activity in the interneurons being studied.

Intracellular recording

Intracellular recordings were taken from the neuropil segments of interneurons in the mesothoracic ganglion (Fig. 1). These interneurons have been previously identified as having monosynaptic excitatory connections from the fSR (Reye and Pearson 1987). The interneurons which were penetrated at least 3 times in both immature and mature locusts are 016 (numbered according to Rowell and Reichert 1991; first described by Rowell and Pearson 1983), 302 and 701 (Robertson and Pearson 1983), 313 and 507 (Reye and Pearson 1987). What we classified as interneuron 016 may in fact not be a single interneuron, but a group of local interneurons which have similar structures. We found it impossible to distinguish these based on morphology. The neuropil segment penetrated was always ipsilateral to the extracellular hook electrode (Fig. 1). Recordings were made with glass microelectrodes filled at the tip with Lucifer Yellow CH (4% in distilled H_2O). The shafts were filled with 0.5 *M* lithium chloride (electrode resistance ~100 MΩ). These are all spiking interneurons and in some cases it was necessary to inject small amounts of hyperpolarizing current to stop the cell from spiking and enable us to measure the EPSPs. After recording EPSPs, hyperpolarizing current was used to fill the impaled cell with Lucifer Yellow. In many instances, prior to removing the microelectrode, the preparation was examined under epifluorescence illumination in order to determine the recording site in the interneuron.

The ganglia were fixed in 4% paraformaldehyde for 1 h, dehydrated in an ethanol series and cleared in methyl salicylate. Ganglia were examined under a compound fluorescence microscope from the dorsal aspect and a drawing of the ganglion's outline and the filled interneuron was made with the aid of a camera lucida. Some interneurons were photographed.

One hundred forty-three penetrations of neurons which received input from the fSR were made. Of these, those which did not show a stable membrane potential after penetration were discarded. Preparations from which the postsynaptic cell was not identifiable, due to insufficient filling with Lucifer Yellow, were not included in the comparison of EPSP parameters. Only flight interneurons which have previously been described as receiving monosynaptic input from the fSR were used in this study. Thus any preparations in which the postsynaptic cell was either a motoneuron or a previously undescribed interneuron were not included in the analysis. This left us with 82 penetrations, 31 from immature and 51 from mature locusts.

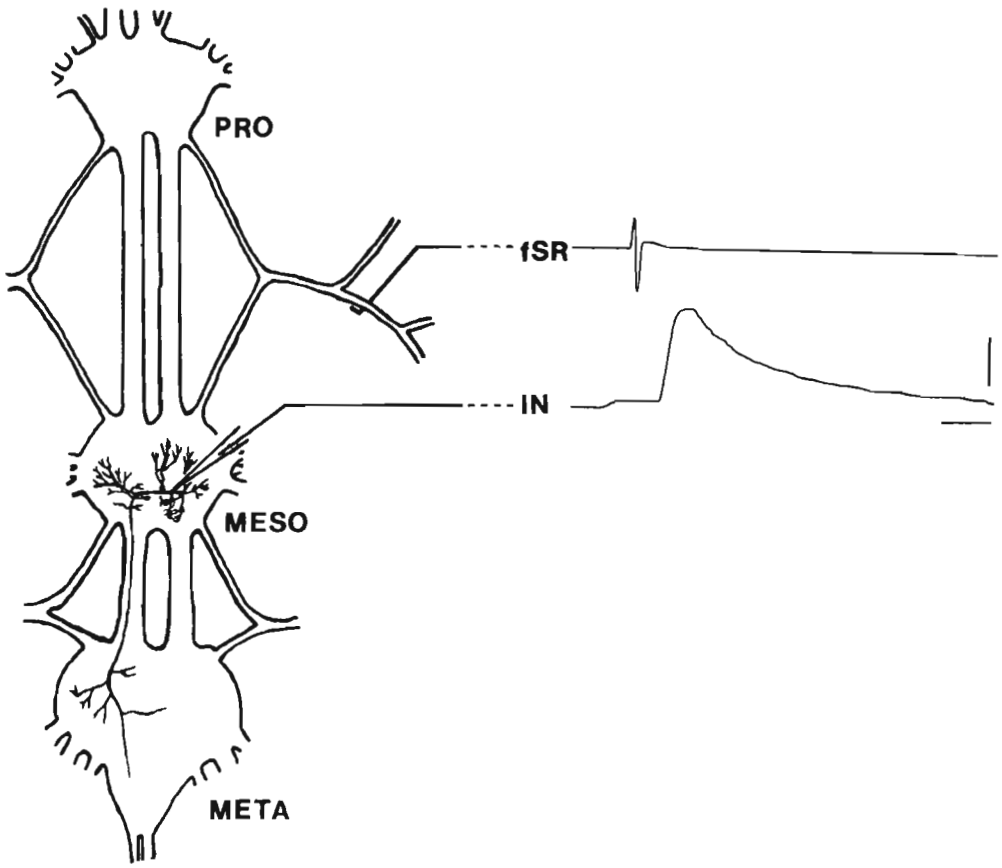

Fig. 1 Schematic diagram of recording situation. The three thoracic ganglia are shown; prothoracic (*PRO*), mesothoracic (*MESO*) and metathoracic (*META*). The extracellular hook electrode is shown placed on mesothoracic nerve 1D with a sample trace of a forewing stretch receptor (*fSR*) action potential. The intracellular microelectrode is shown penetrating the neuropil segment of interneuron 302 ipsilateral to the extracellular electrode with a sample EPSP (*IN*). Vertical scale bar=2 mV, horizontal scale bar=10 ms

Morphological analysis

Diameters of the main neuropil process and the axon of interneuron 302 were measured from photographs. Other morphological parameters were measured from camera lucida drawings of the interneurons and ganglia, using a digitizing tablet and SigmaScan® software (Jandel Scientific, San Rafael, CA). Two-dimensional projected ganglion area was measured by tracing the outline of each drawing. Dendritic projection area was measured by tracing around the perimeter of the high order branches. For interneuron 302 two distances were also measured: 1) the length of the main neuropil process (arrow heads, Fig. 2) from the point where the primary neurite descends to the soma, to the first main branch contralateral to the soma; 2) the distance from the branch point at the main process to the most anterior branches (arrows, Fig. 2). Only those preparations for which the fine higher order branches were well filled, could be used for comparison of the dendritic projection areas. For many of the identified interneurons only 2 preparations at one age were well filled. This precluded statistical analysis using individual neuron types for the most part. All statistical comparisons in Table 1 were made using the unpaired Student's *t*-test.

Analysis of parameters of excitatory post synaptic potentials

Either the spontaneous fSR action potential or the stimulus artefact was used for spike-triggered averaging of the EPSPs. The average trace from at least 25 EPSPs was plotted, along with an extracellular trace, on an x-y plotter. The following parameters were then measured for each average EPSP; the latency from the fSR spike to the onset, the maximum rising slope, the amplitude, the time from onset to the peak, the duration at half amplitude and the area above the baseline. EPSP parameters from immature and mature interneurons were compared using Student's *t*-test.

In some preparations the fSR was stimulated at different frequencies to determine whether frequency of stimulus had an effect on EPSP parameters, and whether this effect was age-related. The nerve containing the fSR was cut distally to prevent spontaneous action potentials. The fSR was stimulated at 1 Hz, 10 Hz, 20 Hz and 30 Hz for about 1 min each. The order in which the frequencies were presented varied and the frequency was returned to 1 Hz. Average EPSP amplitudes, in response to fSR action potentials at 10 Hz, 20 Hz and 30 Hz, were divided by the amplitude at 1 Hz stimulation to normalize the data. No differences in frequency sensitivity were seen with different interneurons. The $\log_{10}$ normalized amplitude was plotted vs. frequency for each individual from which EPSPs were recorded at two or more frequencies in addition to 1 Hz. A linear regression was fit for each individual and the slopes and y-intercepts of the regressions of the immature and mature animals were compared using Student's *t*-test.

A two-way ANOVA was used to test the data in Fig. 5.

Results

Morphology

We found that the projected two-dimensional area of the mesothoracic ganglion increased significantly during post-imaginal maturation (Table 1). This led us to investigate whether the flight interneurons we recorded from were also expanding. Examples of 2 interneurons from immature and mature locusts are shown, interneuron 302 (Fig. 2) and interneuron 701 (Fig. 3). The pooled average of the two-dimensional projection area over which the

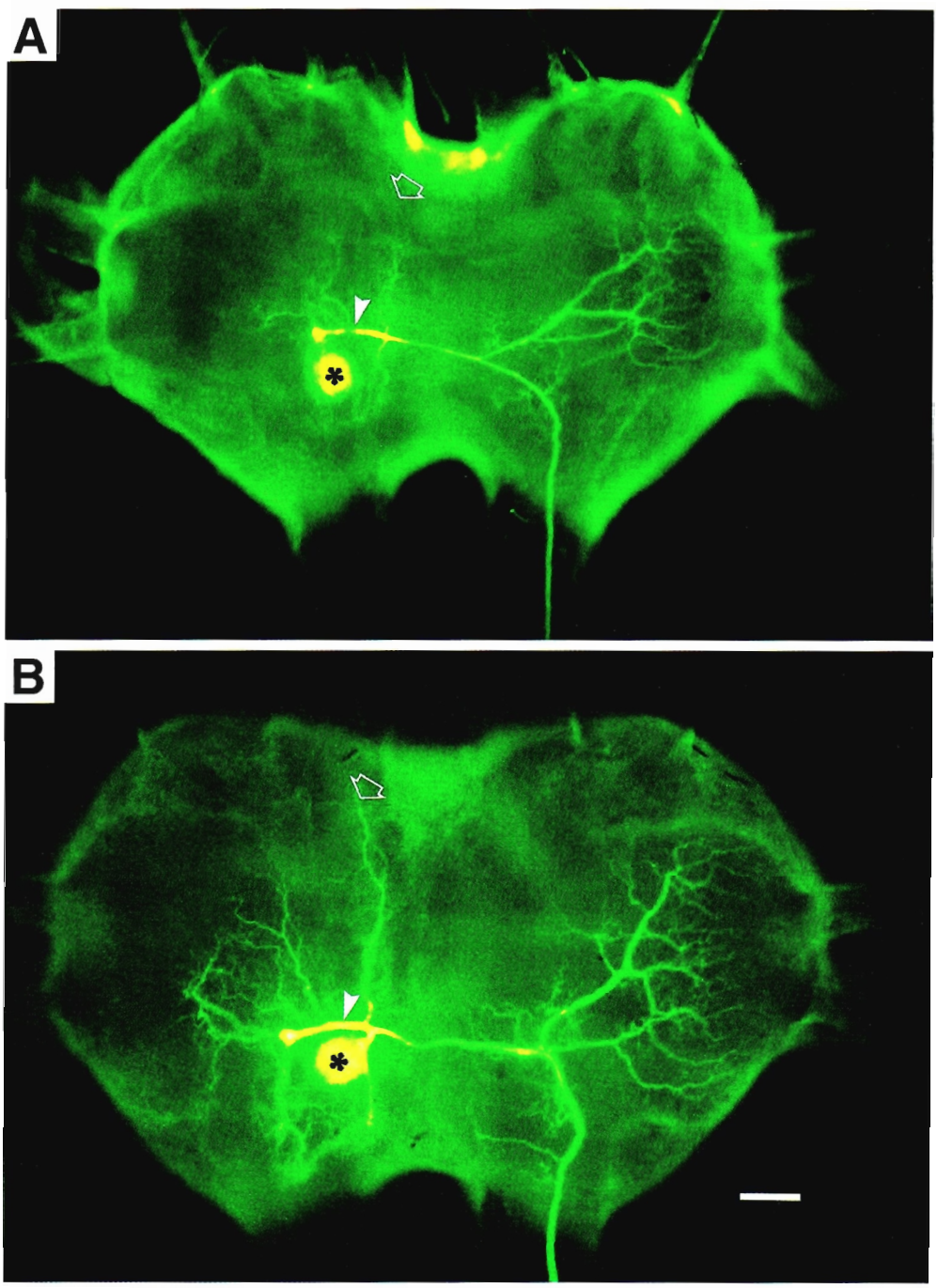

Fig. 2 Photographs of Lucifer Yellow fills of **A** an immature and **B** a mature interneuron 302 in the mesothoracic ganglion taken from the dorsal aspect. The *asterisks* mark the cell bodies which are located ventrally and are out of the plane of focus. There are two distinct areas of dendritic branching, one close to the soma and one where the main neuropil process makes a turn and sends an axon towards the metathoracic ganglion. Note that the main neuropil process appears thicker in the mature interneuron (*arrow heads*, which also mark the intracellular electrode penetration site). The length which the various processes, including the higher order branches extend (*arrows*) appears to be greater in the mature interneuron. Scale bar=100 μm

dendrites branch, increased significantly during adult maturation (Table 1). The dendritic projection area for individual types of interneurons could not usually be compared statistically, due to low numbers of complete fills. For interneurons 016 and 302 there were enough complete fills to compare morphological parameters between immature and mature animals. The increase during maturation of the dendritic projection area for 016 was not significant (Table 1). For 302 there are two distinct areas of dendritic branching; one ipsilateral to the soma, the other contralateral to the soma near the axon (see Fig. 2). These areas were measured separately, and summed for each individual. Both the dendritic projection area near the soma and the total projection area were significantly higher in mature animals (Table 1). The dendritic projection area near the axon was greater in mature individuals (Table 1) but the difference was not significant (P=0.076). The length of both the transverse primary neuropil process (arrow head, Fig. 2), and the distance which the anterior-going branches extend (arrow, Fig. 2) also increased significantly during maturation (Table 1). The diameters of the main neuropil process and the axon of interneuron 302 increased with maturation (Table 1).

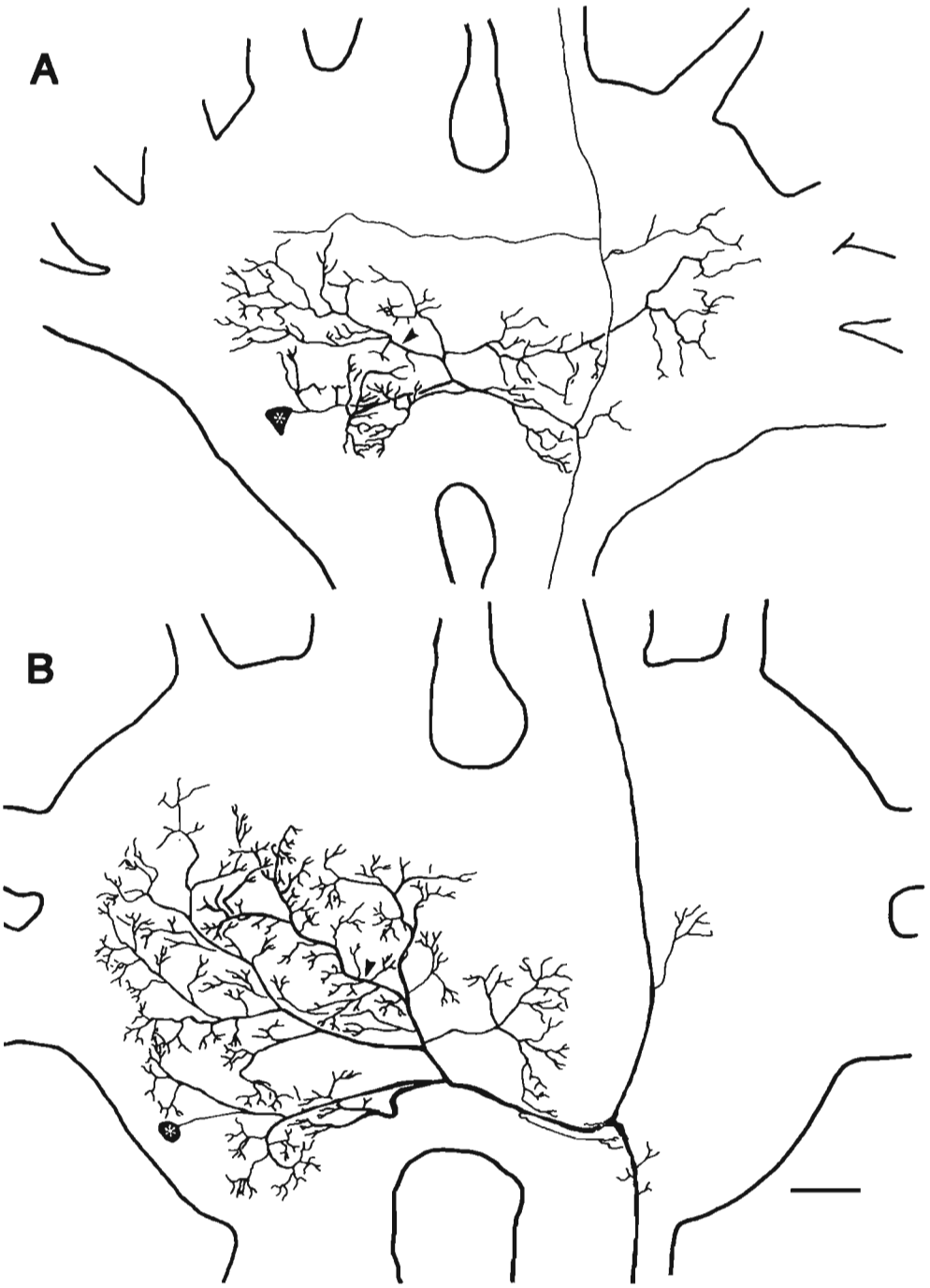

Fig. 3 Two-dimensional camera lucida drawings of interneuron 701 taken from an **A** immature and **B** mature locust. *Asterisks* mark the cell bodies. Note that the processes of the mature interneuron appear more robust and the two-dimensional areas of both the ganglion and the dendritic projections appear greater in the mature locust. *Arrow heads* mark the intracellular electrode penetration site. Scale bar=100 μm

No change in the general architecture of these neurons was noticed with maturation. In Fig. 3, the immature 701 has an anterior branch which crosses the midline of the mesothoracic ganglion while the mature interneuron does not. This was not a maturation-specific difference in branching pattern, but was seen between different examples of this interneuron at both ages.

EPSP parameters

Parameters of EPSPs in identified interneurons were measured from averages of 25 to 100 PSPs triggered from action potentials in the fSR. Parameters were then averaged within the age categories to facilitate comparison between immature and mature locusts. Table 2 shows that for EPSPs recorded from interneuron 302 there were no significant differences, due to maturation, in any of the parameters measured. Interneuron 701 was the only interneuron for which several of the EPSP parameters changed; the amplitude, slope and area all decreased with maturation (Table 2).

These results are interesting in that one would expect the amplitude of EPSPs to decrease if the only change occurring during maturation was growth of the postsy-

Table 1 Comparison of morphological parameters of the mesothoracic ganglion and flight interneurons in immature and mature adult *Locusta migratoria*

Morphological parameter	Interneuron	Immature mean (±SEM; *n*)	Mature mean (±SEM; *n*)	Significance (*t*-test)
Ganglion projection area (mm^2)	–	0.942 (±0.019; *n*=30)	1.132 (±0.023; *n*=31)	*P*<0.001
Dendritic area (total) (mm^2)	all	0.280 (±0.018; *n*=19)	0.388 (±0.025; *n*=24)	*P*<0.05
Dendritic area (total) (mm^2)	016	0.244 (±0.017; *n*=4)	0.297 (±0.021; *n*=7)	n/s
Dendritic area (total) (mm^2)	302	0.284 (±0.008; *n*=5)	0.406 (±0.040; *n*=6)	*P*<0.05
Dendritic area (soma) (mm^2)	302	0.162 (±0.004; *n*=6)	0.239 (±0.020; *n*=7)	*P*<0.05
Dendritic area (axon) (mm^2)	302	0.123 (±0.008; *n*=6)	0.162 (±0.018; *n*=6)	n/s
Neuropil process length (mm)	302	0.378 (±0.010; *n*=8)	0.475 (±0.017; *n*=8)	*P*<0.001
Anterior branch length (mm)	302	0.311 (±0.012; *n*=8)	0.384 (±0.024; *n*=7)	*P*<0.05
Neuropil process diameter (μm)	302	7.0 (±2.7; *n*=5)	11.6 (±2.7; *n*=5)	*P*<0.05
Axon diameter (μm)	302	6.7 (±1.3; *n*=5)	10.3 (±2.4; *n*=5)	*P*<0.05

Table 2 Parameters of EPSPs from the forewing stretch receptor, in identified flight interneurons of immature and mature *Locusta migratoria* (1/2 Amp. Dur., duration at half amplitude)

Neuron	Parameter	Immature mean (±SEM; *n*)	Mature mean (±SEM; *n*)	Significance (*t*-test)
016	Latency (ms)	3.47 (±0.20; *n*=8)	2.80 (±0.15; *n*=14)	*P*<0.05
	Amplitude (mV)	5.57 (±0.67; *n*=8)	3.91 (±0.64; *n*=14)	*n*/s)
	Slope (mV/ms)	3.93 (±1.17; *n*=8)	3.39 (±1.10; *n*=14)	*n*/s
	Time to Peak (ms)	6.26 (±1.02; *n*=8)	5.19 (±0.91; *n*=14)	*n*/s
	1/2 Amp. Dur. (ms)	23.88 (±3.55; *n*=8)	21.62 (±2.91; *n*=14)	*n*/s
	Area (mV*ms)	142.28 (±16.58; *n*=8)	97.80 (±17.62; *n*=13)	*n*/s
302	Latency	2.77 (±0.3; *n*=7)	2.68 (±0.16; *n*=16)	*n*/s
	Amplitude	3.31(±0.82; *n*=7)	2.60(±0.28; *n*=16)	*n*/s
	Slope	3.13(±0.78; *n*=7)	2.28(±0.32; *n*=16)	*n*/s
	Time to Peak	3.08(±0.50; *n*=7)	3.25(±0.44; *n*=16)	*n*/s
	1/2 Amp. Dur.	19.97(±1.52; *n*=6)	21.58(±2.25; *n*=15)	*n*/s
	Area	82.00(±27.95; *n*=6)	55.57(±5.99; *n*=15)	*n*/s
313	Latency	2.92(±0.29; *n*=5)	2.37(±0.25; *n*=7)	*n*/s
	Amplitude	8.56(±1.77; *n*=5)	8.83(±1.39; *n*=7)	*n*/s
	Slope	7.32(±2.11; *n*=5)	7,85(±1.13; *n*=7)	*n*/s
	Time to Peak	3.20(±0.73; *n*=5)	2.53(±0.21; *n*=7)	*n*/s
	1/2 Amp. Dur.	19.78(±4.76; *n*=5)	8.37(±1.95; *n*=7)	*P*<0.05
	Area	195.03(±57.04; *n*=5)	114.62(±18.98; *n*=6)	*n*/s
507	Latency	3.89(±0.57; *n*=6)	2.74(±0.24; *n*=6)	*n*/s
	Amplitude	1.96(±0.48; *n*=6)	1.50(±0.39; *n*=6)	*n*/s
	Slope	1.10(±0.43; *n*=6)	1.02(±0.34; *n*=6)	*n*/s
	Time to Peak	4.97(±0.56; *n*=6)	3.27(±0.25; *n*=6)	*P*<0.05
	1/2 Amp. Dur.	20.92(±1.45; *n*=6)	22.69(±2.10; *n*=6)	*n*/s
	Area	50.49(±15.84; *n*=4)	37.76(±6.07; *n*=6)	*n*/s
701	Latency	2.72(±0.45; *n*=3)	3.16(±0.29; *n*=6)	*n*/s
	Amplitude	8.89(±1.92; *n*=3)	3.04(±0.22; *n*=6)	*P*<0.05
	Slope	6.90(±0.85; *n*=3)	2.46(±0.38; *n*=6)	*P*<0.05
	Time to Peak	2.68(±0.40; *n*=3)	2.85(±0.37; *n*=6)	*n*/s
	1/2 Amp. Dur.	9.59(±0.99; *n*=3)	12.27(±2.56; *n*=6)	*n*/s
	Area	127.00(±29.62; *n*=3)	47.41(±5.77; *n*=6)	*P*<0.05

naptic cell. This, however, was only seen for interneuron 701 (Table 2).

Several factors must be taken into consideration which may have caused or obscured any changes in the PSPs. One is the distance of the recording electrode from the synapses and the relationship to the electrotonic structure of the neuron. We examined the preparations under epifluorescence illumination prior to removing the intracellular electrode. We excluded from further analysis penetrations in which the intracellular electrode did not penetrate the same neuropilar process of each interneuron (indicated with arrow heads in Figs. 2 and 3). Recording from different sites would introduce PSP amplitude differences due to electrotonic length differences which would be unrelated to developmental changes.

Attempts were made to measure the input resistances of the interneurons but these measurements were unreliable. Small diameter Lucifer Yellow-filled electrodes

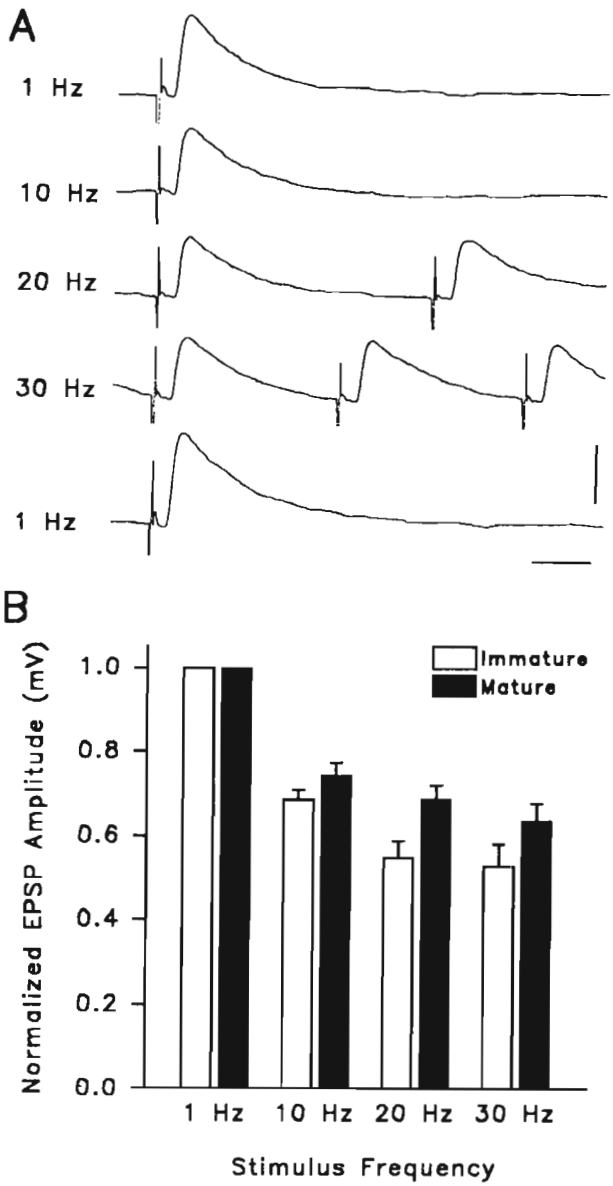

Fig. 4A,B EPSPs depress when the frequency of action potentials in the fSR increases. **A** Average EPSPs from one experiment (mature interneuron 701) while stimulating the fSR at different frequencies. Note that the amplitude at 1 Hz is almost the same before and following high frequency stimulation. **B** Average EPSP amplitudes, normalized to the amplitude at 1 Hz, plotted against frequency of fSR action potentials (*error bars* are standard error of the means). Note that the normalized amplitude of the immature EPSPs is invariably lower than the mature EPSPs. The depression is approximated by an exponential decay. Data obtained from 15 immature and 15 mature interneurons, at least 8 interneurons are represented in each bar. These data are analyzed statistically in Table 3. Vertical scale bar=2 mV, horizontal scale bar=10 ms

have high resistances making it difficult to use either bridge balancing techniques or discontinuous current clamp to obtain an estimate of input resistance in a small process. The hyperpolarizing current injected into some cells to prevent action potentials did not change the size or shape of the PSPs.

Table 3 Y-intercepts and slopes of linear regressions, fit to the $\log_{10}$ transformation of normalized EPSP amplitude versus frequency of fSR stimulation for individual immature and mature *Locusta migratoria*

	Immature Y-intercept	Mature Y-intercept	Immature slope	Mature slope
	0.0146	−0.0058	−0.0165	−0.0134
	−0.0192	0.0025	−0.0095	−0.0080
	−0.0318	−0.0206	−0.0076	−0.0011
	−0.0256	−0.0421	−0.0116	−0.0058
	−0.0192	−0.0405	−0.0149	−0.0009
	−0.0086	−0.0765	−0.0099	−0.0052
	−0.0176	−0.0059	−0.0116	−0.0098
	−0.0340	−0.0550	−0.0123	−0.0061
	−0.0341	−0.0732	−0.0029	−0.0082
	−0.0613	−0.0234	−0.0092	−0.0079
	−0.0461	−0.0270	−0.0112	−0.0070
	−0.0119	–	−0.0180	–
Mean	−0.0246	−0.0334	−0.0113[a]	−0.0067[a]
SEM	0.0056	0.0081	0.0012	0.0011

[a] indicates significant difference, Student's *t*-test

A further consideration is the firing frequency of action potentials in the presynaptic neuron. When we stimulated the stretch receptor at increasing frequencies, we found that EPSPs depressed (Fig. 4). This depression was not long-lasting as the amplitude of EPSPs returned to the starting value at 1 Hz within a few seconds after changing the stimulus frequency. We did not see characteristic differences in the way different types of interneurons depressed. Therefore, average EPSP amplitude at each frequency was divided by the amplitude at 1 Hz to account for differences in individual amplitudes and the data were pooled for each age. Immature EPSPs depressed more than mature EPSPs (Fig. 4B). The depression approximated an exponential decay, therefore, we plotted linear regressions fit to the $\log_{10}$ normalized amplitudes versus the stimulus frequency for each penetration. There was a significant difference in the slopes of the linear regressions between immature and mature EPSP depression (Table 3).

The fSR tends to fire spontaneously at a faster rate in mature locusts (Gray and Robertson 1994). Therefore in order to remove the effects of firing frequency from our comparison, we excluded preparations in which the fSR was either not firing spontaneously between around 1 Hz and less than 3 Hz, or not stimulated at 1 Hz.

Using only the 'select' preparations, that is those where the recording sites were consistent, and the fSR action potentials occurred at or near 1 Hz, the amplitudes of EPSPs were compared (Fig. 5). No change in EPSP amplitude was seen with maturation for interneurons 016, 302 and 313 (Fig. 5). These were the only interneurons, for which we found at least 3 preparations at each age, which met the selection criteria. No significant differences were seen in any of the other parameters measured between immature and mature interneurons of a given type (not shown). There are however, significant differences in EPSP amplitude between neuron types eg.

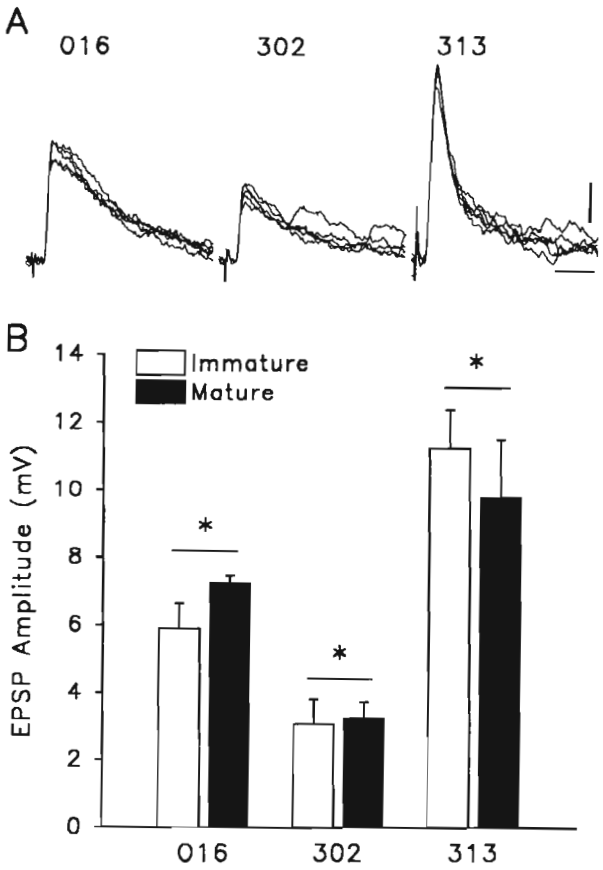

Fig. 5 **A** Overlays of 5 EPSPs in response to stimulation of the fSR at 1 Hz, in mature locusts from interneurons 016, 302 and 313, respectively. **B** Average EPSP amplitudes of the above interneurons from the 'select' preparations (see text). *Error bars* are standard error of the means. No significance differences in EPSP amplitudes were detected due to maturation (*horizontal lines*). Significant differences in EPSP amplitudes were detected between synapses (*asterisks*) (two-way ANOVA). There are 3 to 8 penetrations represented by each bar. Vertical scale bar=2 mV, horizontal scale bar=10 ms

the amplitude of 016 is characteristic and different from the amplitudes of 302 and 313 (Fig. 5).

To see whether there might be some general change in any of the parameters the data from the select preparations were pooled (Fig. 6). The only significant change detected was a decrease in the latencies between the fSR action potential and the EPSP in the mature preparations (Fig. 6). The position of the extracellular electrode varied between preparations, but there was no age-dependent difference in placement, thus we feel that this difference is in fact due to maturation. We also examined latency pooled from all the experiments shown in Table 1. This larger data set also contained a significant decrease in EPSP latency with maturation.

Discussion

The purpose of the present study was to examine whether, during post-imaginal maturation of the locust flight system, there are changes in parameters of identified EPSPs. We found that EPSP parameters do not change during maturation, however, we did find that EPSPs recorded from different interneurons had characteristic amplitudes. There were 3 differences seen with maturation: 1) the latency from an action potential or stimulus artifact in the fSR to onset of EPSPs decreased with maturation; 2) mature EPSPs depressed less than immature EPSPs when the presynaptic cell was stimulated at high frequencies; 3) the size of the postsynaptic cell increases with maturation.

During post-imaginal maturation of the locust, we found significant growth both of the two-dimensional projected area of the mesothoracic ganglion and of the postsynaptic cells. This agrees with previous studies in hemimetabolous insects, which have shown growth of the mesothoracic ganglion well into adulthood in *Schistocerca* (Sbrenna 1971), and in *Locusta migratoria* (Gray and Robertson 1994). This expansion is due largely to an increase in the volume of the neuropil (Afify 1960; Sbrenna 1971). Gymer and Edwards (1967) showed that the number of neurons in the last abdominal ganglion of the cricket *Acheta domesticus* do not increase following hatching. A subsequent forty-fold increase in ganglion volume is due primarily to increasing neuronal cell volume (Gymer and Edwards 1967; see also Sbrenna 1971 for *Schistocerca*). This is in contrast to *Manduca*, a holometabolous insect, in which postembryonic neurogenesis has been demonstrated (Booker and Truman 1987). We did not test for the presence of neurogenesis in the locust. However, the interneurons we examined showed increases in the area over which their processes extend, and in the length of processes during adult maturation. The diameters of both the neuropil process, which the intracellular electrode penetrated, and the axon of interneuron 302 also increased with maturation.

This growth may have important implications for the flight circuitry. The increase in diameter of axonal processes should result in an increase in the conduction velocity of action potentials in flight interneurons. In the locust fSR and the dorsal longitudinal motoneuron, conduction velocity increases during adult maturation and may partly account for the increase in wingbeat frequency (Gray and Robertson 1994). An increase in conduction velocity of flight interneurons may also help speed up the central component of the flight rhythm generator, particularly with respect to those interneurons which communicate between ganglia.

Increasing diameters of dendritic processes will also result in a decrease in the input resistance of the interneuron. EPSPs recorded at sites electrically distant from the synapse will therefore be smaller in amplitude unless there is some compensatory increase in either the synaptic current or synaptic density (Lev-Tov et al. 1983; Jack et al. 1983). Since we did not find that EPSP amplitudes decreased during this period of growth, there must be some compensation in the system which keeps parameters of EPSPs constant during adult maturation. Goldfish retinal ganglion cells grow throughout the animal's life

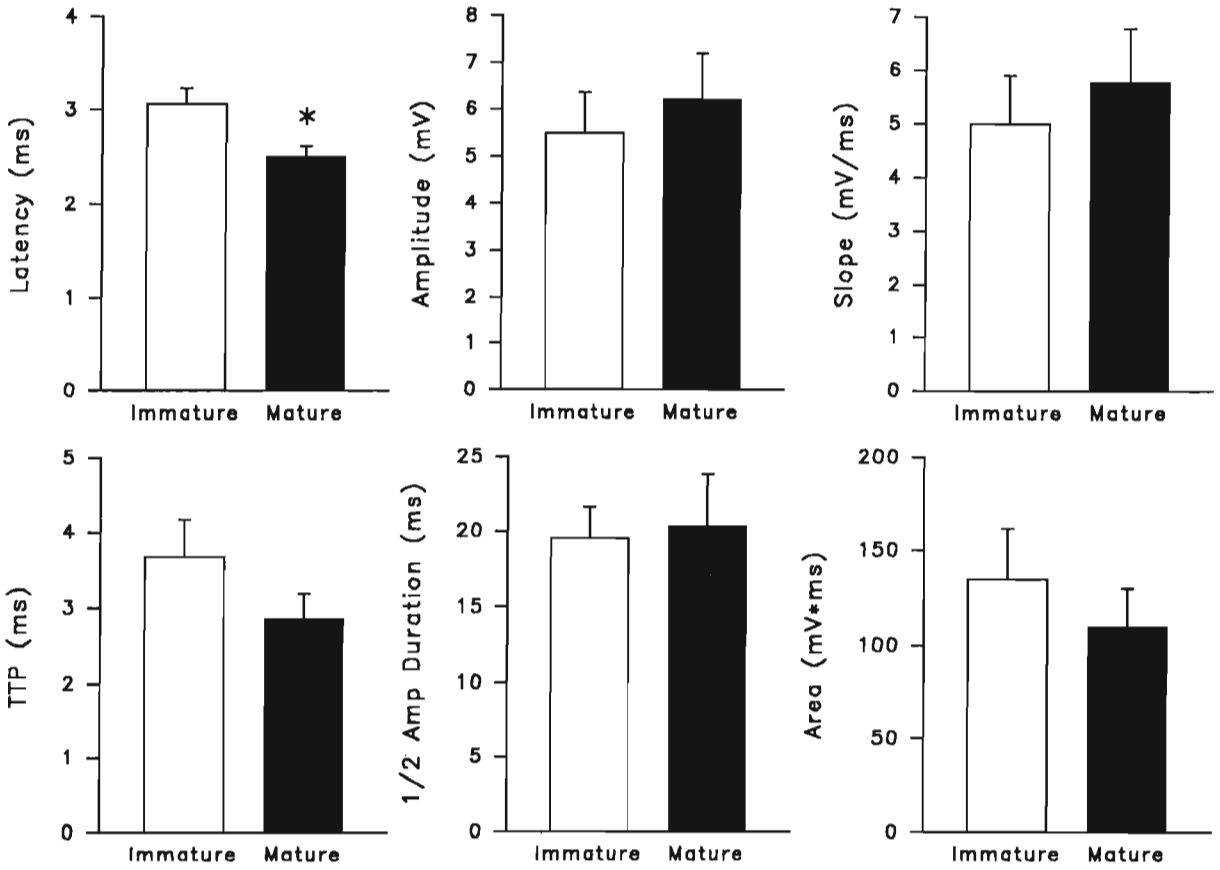

Fig. 6 Average EPSP parameters and standard error bars from the 'select' immature and mature preparations. Latency decreases significantly with maturation (*asterisk*). None of the other parameters show a significant difference between immature and mature EPSPs. Data obtained from 17 immature and 15 mature interneurons

and presumably maintain their signalling properties despite the predicted decrease in input resistance (Bloomfield and Hitchcock 1991). An increase in the number of synaptic contacts found on large arbors may allow the amplitude of PSPs to remain constant (Hitchcock 1993). In the cricket cercal system, there is rearrangement of sensory neuron-to-interneuron synapses that is thought to maintain interneuron response properties as the number and size of sensory receptors increase during development (Chiba et al. 1992). The strength of some identified synapses systematically decrease during development while others increase in strength over the course of several instars (Chiba et al. 1988). In the locust flight system synaptic rearrangement may also occur and continue past the imaginal moult to compensate for growth of the interneurons and to maintain the strength of the connection from the fSR. An alternative mechanism whereby EPSP size could be maintained in a growing postsynaptic cell could be an increase in the current generated at each postsynaptic site as has been demonstrated in the crustacean neuromuscular junction (Lnenicka and Mellon 1983). Such a mechanism could be effected by changes in either pre- or postsynaptic properties.

The fSR firing frequency was found to depress the EPSPs. The EPSPs in immature interneurons were more sensitive to stimulus frequency than mature EPSPs. Terminal processes may have smaller diameters in the immature locusts increasing the chance of conduction failure of high frequency action potentials to the presynaptic terminals. Alternatively, if the rate of supply of transmitter is limited in the immature animals, there may be depletion of available transmitter as frequency increases. Changes in the responsiveness of the postsynaptic membrane could also be involved. We are not yet able to determine which, if any, of the possibilities presented here account for the different depression patterns in immature and mature locusts.

When comparing only those preparations where the penetration sites, in a given type of interneuron, were consistent and where the stimulus frequency was around 1 Hz, we were able to distinguish significant differences in EPSP amplitude between types of interneurons. The difference in amplitudes of EPSPs from different interneurons, in response to an action potential in the same presynaptic cell, may indicate several things. One is that the electrotonic distances, of the recording sites, from the synapses varied drastically from cell to cell. This is probably not the primary cause of the differences we found between neuron types. EPSPs recorded simultaneously from two parts of a flight motoneuron do not differ much in amplitude (Schneider 1991). Also, it is known that single local interneurons in *Schistocerca*

americana make central inhibitory synapses with different parameters, onto different postsynaptic neurons (Laurent and Sivaramakrishnan 1992). Quantal analysis revealed that quantal content at different contacts from the same presynaptic cell differed, suggesting that release probabilities were different between the sites associated with different postsynaptic cells (Laurent and Sivaramakrishnan 1992). Gardner (1991) has shown that in *Aplysia* buccal ganglia, synaptic current amplitudes are different for synapses in different targets from a single presynaptic neuron. This was also found to be due to presynaptic factors (Gardner 1991). Thus the differences in EPSP amplitudes which we found are likely not due only to differences in the electrotonic structure of these interneurons, but probably indicate specialization at the level of individual synapses.

The only change in EPSP parameters seen with maturation was the decrease in latency from the fSR spike to the onset of the EPSP. The position of the extracellular electrode varied only slightly from preparation to preparation, and there was no age-dependent bias. Significant differences in latency were only apparent when a large number of trials were combined. It is likely that the decrease in latency seen here is due to the increase in conduction velocity of the fSR (Gray and Robertson 1994). The EPSPs travel electrotonically from the synapse to the recording electrode and would therefore be recorded almost instantaneously following onset, regardless of age. We cannot rule out the possibility that synaptic delay may change with maturation as well, but we could not, with the paradigm used, determine what the relative contributions of conduction velocity and synaptic transmission were to the change in latency measured.

The problem we set out to investigate was whether changes in the interactions between afferents and interneurons in the flight system occur during post-imaginal maturation. We have not found a change in parameters of identified EPSPs from our population of flight interneurons. This does not preclude that other EPSPs or IPSPs may change in efficacy during adult maturation, but does indicate that there is neither a general strengthening nor weakening of synaptic efficacy.

We did however, detect two changes which may bear some influence on wingbeat frequency. The first is the decrease in latency. This is most likely due to an increase in conduction velocity of the presynaptic cell but as the flight system contains mainly spiking interneurons, a general increase in diameters and consequent increase in conduction velocities could effect a 'speeding-up' of the circuitry. The reduced depression seen at higher frequencies in the mature animals may also affect output of the flight motor. That is, in an immature animal the output frequency of the central circuitry may be limited by EPSP amplitudes which become less efficacious at high frequencies. As the animal matures and EPSP amplitude becomes less frequency-sensitive, a higher output frequency can be supported. The effects of maturation seen in the central circuitry are accompanied by changes in peripheral components which would act in concert to produce maturation of the flight system.

In conclusion then, it appears that a mechanism exists which allows EPSP parameters, especially amplitude, to remain constant during growth of the central nervous system. EPSP amplitudes are characteristic in different flight interneurons even though the presynaptic cell is the same. If the difference in frequency-sensitivity of immature and mature EPSP amplitudes is common to all synapses it may account for the central component of the increase in wingbeat frequency during post-imaginal maturation.

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References

Afify AM (1960) Über die postembryonale Entwicklung des Zentralnervensystems (ZNS) bei der Wanderheuschrecke *Locusta migratoria migratorioides* (R. & F.) (Orthoptera-Acrididae). Zool Jb 78:1–38

Altman JS, Tyrer NM (1977) The locust wing hinge stretch receptors I. Primary sensory neurones with enormous central arborizations. J Comp Neurol 172:409–430

Bekoff A, Nusbaum MP, Sabichi AL, Clifford M (1987) Neural control of limb coordination I. Comparison of hatching and walking motor output patterns in normal and deafferented chicks. J Neurosci 7:2320–2330

Bloomfield SA, Hitchcock PF (1991) Dendritic arbors of large field ganglion cells show scaled growth during expansion of the goldfish retina. J Neurosci 11:910–917

Booker R, Truman JW (1987) Postembryonic neurogenesis in the CNS of the tobacco hornworm, *Manduca sexta* I. Neuroblast arrays and the fate of their progeny during metamorphosis. J Comp Neurol 255:548–559

Burrows M (1975) Monosynaptic connexions between wing stretch receptors and flight motoneurones of the locust. J Exp Biol 62:189–219

Campbell TI (1961) The anatomy of the nervous system of the mesothorax of *Locusta migratoria migratorioides* (R. and F.). Proc Zool Soc (Lond) 137:403–432

Chiba A, Shepherd D, Murphey RK (1988) Synaptic rearrangement during postembryonic development in the cricket. Science 240:901–905

Chiba A, Kämper G, Murphey RK (1992) Response properties of interneurons of the cricket cercal sensory system are conserved in spite of changes in peripheral receptors during maturation. J Exp Biol 164:205–226

Gardner D (1991) Presynaptic transmitter release is specified by postsynaptic neurons of *Aplysia* buccal ganglia. J Neurophysiol 66:2150–2154

Gettrup E (1962) Thoracic proprioceptors in the flight system of locusts. Nature (Lond) 193:498–499

Gray JR, Robertson RM (1994) Activity of the forewing stretch receptor in immature and mature adult locusts. J Comp Physiol A (in press)

Gymer A, Edwards JS (1967) The development of the insect nervous system I. An analysis of postembryonic growth in the terminal ganglion of *Acheta domesticus*. J Morphol 123:191–197

Harris-Warrick RM, Marder E (1991) Modulation of neural networks for behavior. Annu Rev Neurosci 14:39–57

Hitchcock PF (1993) Mature, growing ganglion cells acquire new synapses in the retina of the goldfish. Visual Neurosci 10:219–224

Jack JJB, Noble D, Tsien RW (1983) Electric current flow in excitable cells. Clarendon Press, Oxford
Jacobs GA, Weeks JC (1990) Postsynaptic changes at a sensory-to-motoneuron synapse contribute to the developmental loss of a reflex behavior during insect metamorphosis. J Neurosci 10:1341–1356
Kent KS, Levine RB (1993) Dendritic reorganization of an identified neuron during metamorphosis of the moth *Manduca sexta*: The influence of interactions with the periphery. J Neurobiol 24:1–22
Kutsch W (1974) The influence of the wing sense organs on the flight motor pattern in maturing adult locusts. J Comp Physiol 88:413–424
Laurent G, Sivaramakrishnan A (1992) Single local interneurons in the locust make central synapses with different properties of transmitter release on distinct postsynaptic neurons. J Neurosci 12:2370–2380
Lev-Tov A, Miller JP, Burke RE, Rall W (1983) Factors that control amplitude of EPSPs in dendritic neurons. J Neurophysiol 50:399–412
Levine RB, Truman JW (1982) Metamorphosis of the insect nervous system: changes in the morphology and synaptic interactions of identified neurons. Nature (London) 299:250–252
Lisman JE, Harris KM (1993) Quantal analysis and synaptic anatomy – Integrating two views of hippocampal plasticity. Trends Neurosci 16:141–147
Lnenicka GA, Mellon DeF (1983) Changes in electrical properties and quantal current during growth of identified muscle fibres in the crayfish. J Physiol (Lond) 345:261–284
McNaughton BL (1993) The mechanism of expression of long-term enhancement of hippocampal synapses: Current issues and theoretical implications. Annu Rev Physiol 55:375–396
Nottebohm F (1981) A brain for all seasons: cyclical anatomical changes in song control nuclei of the canary brain. Science 214:1368–1370
Pabst H (1965) Elektrophysiologische Untersuchungen des Streckrezeptors am Flügelgelenk der Wanderheuschrecke *Locusta migratoria*. Z Vergl Physiol 50:498–541
Reye DN, Pearson KG (1987) Projections of the wing stretch receptors to central flight neurons in the locust. J Neurosci 7:2476–2487
Reye DN, Pearson KG (1988) Entrainment of the locust central flight oscillator by wing stretch receptor stimulation. J Comp Physiol A 162:77–89
Robertson RM, Pearson KG (1982) A preparation for the intracellular analysis of neuronal activity during flight in the locust. J Comp Physiol 146:311–320
Robertson RM, Pearson KG (1983) Interneurons in the flight system of the locust: Distribution, connections, and resetting properties. J Comp Neurol 215:33–50
Robertson RM, Pearson KG (1984) Interneuronal organization in the flight system of the locust. J Insect Physiol 30:95–101
Rowell CHF, Pearson KG (1983) Ocellar input to the flight motor system of the locust: Structure and function. J Exp Biol 103:265–288
Rowell CHF, Reichert H (1991) Mesothoracic interneurons involved in flight steering in the locust. Tissue Cell 23:75–139
Sanes DH (1993) The development of synaptic function and integration in the central auditory system. J Neurosci 13:2627–2637
Sbrenna G (1971) Postembryonic growth of the ventral nerve cord in *Schistocerca gregaria* (Orthoptera: Acrididae). Boll Zooll 38:49–74
Schneider H (1991) Untersuchungen zur funktionellen Differenzierung von Motoneuronen bei Heuschrecken. PhD Thesis, Universität Konstanz
Stevenson PA, Kutsch W (1986) Basic circuitry of an adult-specific motor program completed with embryogenesis. Naturwissenschaften 73:741
Stevenson PA, Kutsch W (1988) Demonstration of functional connectivity of the flight motor system in all stages of the locust. J Comp Physiol A 162:247–259
Weeks JC, Jacobs GA, Miles CI (1989) Hormonally mediated modifications of neuronal structure, synaptic connectivity, and behavior during metamorphosis of the tobacco hornworm, *Manduca sexta*. Am Zool 29:1331–1344
Wolf H, Pearson KG (1987) Comparison of motor patterns in the intact and deafferented flight system of the locust. II. Intracellular recordings from flight motoneurons. J Comp Physiol A 160:269–279