



Co-ordination of the Flight Motor Pattern with Forewing Stretch Receptor Stimulation in Immature and Mature Adult Locusts

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ABSTRACT. We investigated the effect of proprioceptive feedback on the flight motor pattern during maturation of the locust flight system by stimulating the forewing stretch receptors (fSRs) in immature and mature adults and recording the frequency of wing depressor muscle activity. Stimulation of both fSRs produced co-ordination with the motor pattern in 4 of 10 immature animals and in 6 of 16 mature animals. The strength of co-ordination was measured as the variability of the phase of hindwing depressor muscle activity within the stimulus period. Co-ordination was stronger and the phase values higher in mature animals presented with a stimulus that mimics fSR activity in response to rhythmic wing elevation. We conclude that maturation of the flight motor is due, in part, to a change in the response of the central circuitry to a change in the timing of proprioceptive input. COMP BIOCHEM PHYSIOL 118A;1:125–130, 1997. © 1997 Elsevier Science Inc.

KEY WORDS. Locust, flight, development, maturation, motor pattern, proprioception, entrainment, stretch receptor

INTRODUCTION

Many of the studies that describe the operation of motor pattern generators come from preparations of fully mature animals in which the circuitry and behaviour are established. There are, however, relatively fewer studies that examine how the fully mature pattern is attained [see, e.g., (2,3,8,23)]. The flight system of the locust is an invaluable model for studying the operation and ontogeny of rhythmical motor pattern generators since the circuitry consists of relatively few, well described, neurons that can produce a relatively complex behaviour (22).

Maturation of the locust flight system occurs over the first 2 weeks of adult life and involves an exponential increase of the wingbeat frequency to a maintained level. The developmental increase in wingbeat frequency is not influenced by flight experience (13,14) or by having the wings fixed throughout maturation (15). Moreover, deafferentation immediately following imaginal ecdysis does not prevent a subsequent increase in the wingbeat frequency (12,13,24) suggesting that proprioceptive input from the wings is not required for the normal maturational increase in wingbeat frequency. However, the irregular motor pattern observed in immature animals may be due to distorted sensory feedback when the wings are less rigid (1). Previously, we have

shown that the difference between the rhythm frequencies of acutely deafferented mature and immature animals is not as great as the difference between those of intact mature and immature animals (11). Moreover, at higher (e.g., mature) wingbeat frequencies sensory feedback plays a larger role in generating the motor pattern than at lower (e.g., immature) wingbeat frequencies (29). Thus, since the wingbeat frequency increases during maturation, the efficacy of sensory feedback may also increase during this time.

Recently, it has been shown that removal of a hindwing proprioceptor, the tegula, results in an initial decrease in the flight rhythm frequency (5,6). Over the next 2 weeks the wingbeat frequency increases to near pre-ablation levels, and this may be mediated by sprouting of the metathoracic branches of the forewing tegula afferents, resulting in new synapses forming with interneurons that normally receive input from the hindwing tegulae (5). Moreover, the flight circuitry may have the capacity to be remodelled throughout adult life (9) to allow it to cope with the changing weight of the animal or with external perturbations. Thus, previous findings (12,13) may have been confounded by a period of recovery during maturation.

One of the wing proprioceptors, the wing hinge stretch receptor (SR), is a single cell located at the base of each wing (27) that monitors wing position (20). The axon of the SR projects into the three thoracic ganglia and makes connections to flight motoneurons (4) and interneurons (19). Many studies have demonstrated that the phasic activity of the stretch receptor can entrain the rhythm frequency

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of mature locusts (10,18,19,21,25–27,29). Thus, the stretch receptor system is a particularly attractive model for examining the influence of proprioceptive feedback during motor pattern development.

Although deafferentation experiments suggest that proprioceptive feedback is not involved in flight maturation (16), there have been no direct measurements of the effects of proprioceptive activity during this time. During maturation, stretch receptor activity increases in response to the same amplitude of rhythmic wing elevation (11). In this paper we describe experiments designed to test the idea that these changes in fSR activity are instrumental in stabilizing the motor pattern and perhaps increasing the wingbeat frequency. If the pattern of fSR activity has control over the motor pattern then the same fSR pattern should be equally co-ordinated with immature and mature motor pattern generators. Also one would expect an immature fSR pattern to produce less effective co-ordination with a mature motor pattern.

MATERIALS AND METHODS

Adult *Locusta migratoria* were obtained from a colony (31°C, 18:6 light:dark) maintained at the Department of Biology at Queen's University. Animals were selected at 0–2 days (immature) or 14 days (mature) after imaginal ecdysis. Experiments were carried out at room temperature (approximately 25°C).

It has been shown that stimulation of both fSRs can induce phase-locking when a stimulus is presented to an operating flight motor pattern (21). Therefore, we used a similar preparation to examine the phase-locking ability of the fSR during maturation (Fig. 1). After the legs were removed, the locust was mounted ventral-side up and a small window of cuticle was removed to expose the prothoracic and mesothoracic ganglia, as well as mesothoracic nerve 1 (Meso N1) and prothoracic nerve 6 [Pro N6, branches named according to (7)]. Monopolar hook electrodes were placed on both right and left Pro N6. The only sensory afferent in this branch comes from the fSR (19). The segment of Pro N6 between the electrode and the prothoracic ganglion was crushed and Meso N1 was cut distal to the branch with Pro N6. Crushing Pro N6 prevented antidromic stimulation of motoneurons in the prothoracic ganglion whose axons extend out Pro N6 whereas cutting Meso N1 distal to the branch with Pro N6 blocked input from forewing sensory organs during experimental trials. The hook electrodes and nerve were insulated with a mixture of vaseline and mineral oil. The window was also covered with vaseline to prevent the underlying tissue from drying out. An EMG electrode was placed in a hindwing first basalar muscle to monitor the flight motor pattern. Stimuli were applied to both fSRs using a Grass S88 two channel stimulator. The threshold for fSR stimulation was determined initially by placing a recording electrode on Meso N1 ipsilateral to the

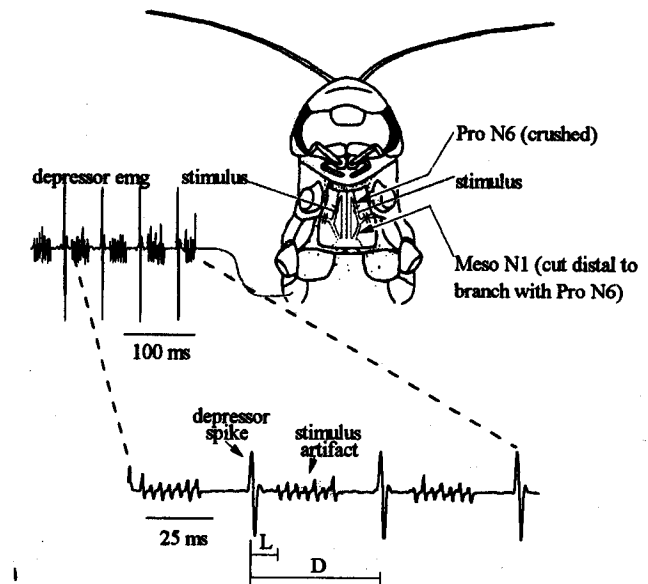


FIG. 1. Setup for measuring entrainment of the flight motor pattern by the forewing stretch receptor [modified from (19)]. Crushing both prothoracic nerves 6 (Pro N6) between the stimulus electrodes and the prothoracic ganglion as well as cutting both mesothoracic nerves 1 (Meso N1) distal to the branch with Pro N6 allowed for selective stimulation of the forewing stretch receptor axon. The expanded portion of the depressor EMG shows the timing of the stimulus within the depressor period (D). Stimulus trains are represented here as a stimulus artifact within the depressor EMG signal. Actual measurements of stimulus onset were measured from a separate channel from the stimulus monitor output. The phase of the stimulus was determined by dividing the latency of depressor activity to stimulus onset (L) by D.

stimulating electrode on Pro N6. Since the centrally projecting axon of the fSR is the only afferent axon that branches into Meso N1 and Pro N6, it is possible to selectively stimulate the fSR in Pro N6 and record the resulting activity in Meso N1. The threshold for inducing action potentials in the fSR was between 0.7 and 1.8 V which is consistent with previous findings (19). Stimulating voltages above threshold produced a noticeable twitching of the wings due to current spread and coactivation of motoneurons supplying the flight muscles. Thus, for subsequent experiments, the stimulating voltage was set to a value just below that which produced wing twitching and was consistently within the normal range for fSR activation. The stimulus voltage was checked often throughout the experiment to insure that flight muscles were not coactivated.

Immature ($n = 10$) and mature ($n = 16$) animals were presented with two different stimulus regimes: 1) single pulses (0.1 ms duration) or 2) trains of single pulses (25 ms duration, 275 Hz intraburst frequency). These levels approximate immature and mature levels of fSR activity, respectively, during normal wing elevations (11). The initial rhythm frequency was measured from the depressor EMG to determine the range of stimulus frequencies to be used.

Stimuli were presented after flight initiation and ranged from 2 Hz below to 8 Hz above (incremented by 1 Hz) the initial rhythm frequency. Thus there were 11 trials per stimulus type for each animal for a total of 572 trials.

Co-ordination of depressor activity with the stimulus was determined by measuring the period between the depressor spike and the stimulus pulse or the beginning of a stimulus train and the depressor spike (L) and depressor period (D) (see, e.g., Fig. 1). The phase of the stimulus within the depressor cycle was determined by dividing L by D. The flight rhythm was considered to be co-ordinated with the stimulus if the difference between consecutive phase values was 0.1 phase units or less for at least 20 cycles and there was a measurable change in the depressor frequency such that it matched the stimulus frequency. There are three types of interactions between an oscillator and an applied rhythmical stimulus (26). If the oscillator maintains its natural frequency in the presence of the stimulus then there is no co-ordination. If the oscillator is synchronized to the stimulus then there is absolute co-ordination (i.e., entrainment). If the oscillator frequency, in the presence of the stimulus, lies between the stimulus frequency and the natural oscillator frequency then there is relative co-ordination. That is, the stimulus has an effect on the oscillator yet the oscillator is not perfectly entrained. The variability of the oscillator frequency during relative co-ordination can thus provide an indication of the strength of the effect that the stimulus has on the oscillator.

The variability of the depressor frequency and the phase of the stimulus within the depressor period was quantified for each respective trial as the coefficient of variation (V), where $V = (\text{standard deviation}/\text{mean}) \times 100\%$. The mean coefficient of variation for each data set was compared between immature and mature trials.

All recordings were stored on video tape and later digitized and analyzed using Datawave Technologies acquisition and off-line analysis software and a custom written IBM Quickbasic program. Significant differences were assessed using a Student's *t*-test. Samples were considered to be significantly different at $p < 0.05$.

RESULTS

Four of 10 immature locusts tested, and 6 of 16 mature locusts tested showed co-ordination between the stimulus and depressor frequencies that satisfied the criteria described in Materials and Methods. Figure 2 shows sample data from a sequence from a mature animal in which the stimulus showed absolute co-ordination with the depressor cycle. The animal was presented with pulse trains at 18.3 Hz and the instantaneous phase values (upper graph) and depressor frequencies (lower graph) are shown. When the stimulus was turned on, there was an initial, brief period of relative co-ordination over the first 2 sec followed by a 6-sec period in which the stimulus and depressor were not co-ordinated.

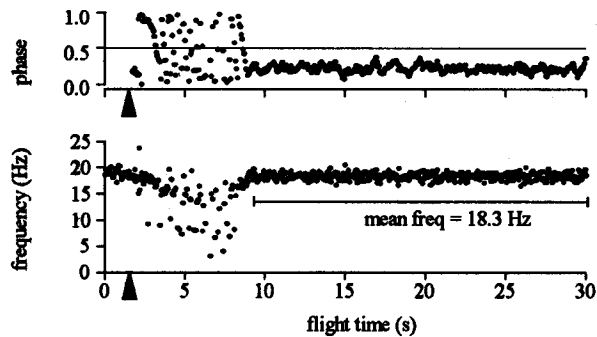


FIG. 2. Sample data from a mature animal. When the stimulus is turned on there is an initial, brief period of phase drifting over the next 2 sec which then becomes uncoupled. At about 8 sec into the flight sequence, the motor pattern became phase-locked to the stimulus and remained so throughout the rest of the sequence.

During this time, the depressor frequency decreased and ranged from less than 5 Hz to about 20 Hz. The lower frequencies shown in this example (5–10 Hz) were due to “missing” depressor spikes. Thus subsequent measurements of the depressor frequency would be one-half of the actual value. This lasted for only about 20 cycles, after which the EMG signal was stabilized for the remainder of the flight sequence. We observed this even in relatively intact animals, an observation also reported for elevator muscles (28) and thus it is not an effect of the dissection. Interestingly, in the example shown, this effect disappeared as the depressor frequency became phase-locked to the stimulus frequency, about 8 sec after stimulus onset. This provides further evidence that the stimulus induced better co-ordination within the flight system. The mean depressor frequency during entrainment was 18.3 ± 0.03 Hz, which was the same as the stimulus frequency of 18.3 Hz (i.e., absolute co-ordination). Of the four immature animals that showed co-ordination, three were co-ordinated only with a single pulse whereas of the six mature animals that showed co-ordination, five were co-ordinated only with a train of pulses.

Absolute co-ordination of the flight motor with the fSR implies that the rhythm frequency (i.e., depressor frequency) is equal to the stimulus frequency. Thus, plotting a range of stimulus frequencies against the corresponding depressor frequencies should produce a straight line with a slope of one. Figure 3A shows that during periods of co-ordination the depressor frequency matched the stimulus frequency. The variability of the depressor frequency during this time was similar in immature and mature locusts (Fig. 3B). The mean phase of the stimulus within the depressor period during these sequences, however, was significantly higher in immature animals than in mature animals (Fig. 4A). That is to say that during maturation depressor activity is delayed within the stimulus period. Moreover, the phase value is more variable in immature animals (Fig. 4B), suggesting that there is a tighter coupling between the “ma-

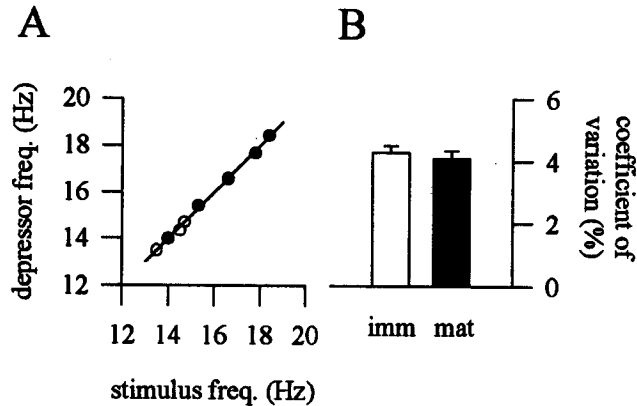


FIG. 3. Comparison of co-ordination parameters between immature ($n = 3$) animals presented with a single pulse and mature ($n = 5$) animals presented with pulse trains. Data shown are from trials that showed clear co-ordination between the stimulus and the depressor activity. (A) Plot of stimulus frequency versus depressor frequency. (B) The variability of the depressor frequency during these trials was similar in immature and mature animals. Bars represent mean \pm SEM.

ture" stimulus and the mature depressor cycle than there is between the "immature" stimulus and the immature depressor cycle. It is possible that a decrease in the phase of the stimulus at a time when the depressor period is decreasing may result in a constant delay between the depressor strike and the stimulus. This suggests that the absolute timing of stimulus and depressor would be constant. However, as shown in Fig. 4C, there is a significant increase in the depressor to stimulus interval, demonstrating that timing of depressor activity within the stimulus period changes relatively and absolutely during maturation.

The single immature animal that was co-ordinated with a mature stimulus had a phase value of 0.72 whereas the single mature animal that was co-ordinated with an immature stimulus had a phase value of 0.31 (data not shown). These phase values were similar to those of aged matched animals presented with the "appropriate" stimulus. The coefficient of variation for these animals was 16.8 and 17.4%, respectively.

DISCUSSION

Stimulation of both fSRs with either single pulses or high frequency bursts can co-ordinate the flight rhythm in immature and mature adult locusts (Fig. 3). However, of the four immature animals that showed co-ordination, three were co-ordinated only with a single pulse stimulus whereas of the six mature animals that showed co-ordination, five were co-ordinated only with pulse trains. Given that the immature fSR responds to rhythmic wing elevation with a single spike and the mature fSR responds with a burst of spikes (11), the above findings suggest that co-ordination is

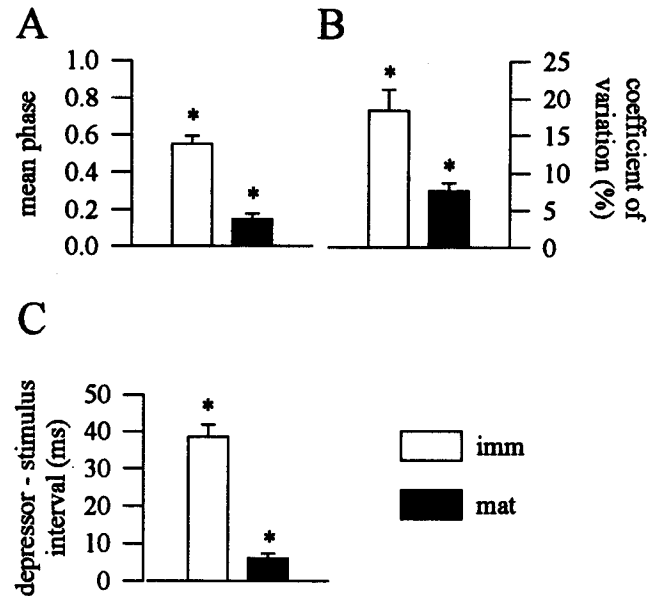


FIG. 4. The phase of the stimulus within the depressor cycle of immature animals is significantly higher than (A) and more variable than (B) that in mature locusts. (C) Concomitant with the decrease in phase, there is a decrease in the depressor-stimulus interval during maturation. Bars represent mean \pm SEM. *Indicates a significant difference using a Student's *t*-test at $p < 0.05$.

stronger when the flight motor is presented with the "proper" level of proprioceptive feedback.

Co-ordination was not always established at stimulus onset. A similar delay was also observed in previous studies (17,25,26). This delay may be explained by the relative timing of the stimulus within the depressor at stimulus onset (26). If the stimulus starts outside the preferred phase then co-ordination may not occur until there is some perturbation that shifts the depressor period. Once the depressor cycle is entrained to the stimulus cycle, the flight motor will remain phase-locked until some other perturbation affects the depressor cycle.

In this study we found that co-ordination was stronger if the stimulus frequency was within 1.5–2 Hz (or 10–13%) of the depressor frequency (data not shown). It has also been shown that entrainment occurs provided the stimulus frequency does not differ from the depressor frequency by more than 10–15% (26). Two important findings in this paper were that the rhythm frequency of immature animals could not be elevated to mature levels even when presented with "mature" levels of fSR activity and that mature animals can be entrained over a greater relative range of stimulus frequencies. These results suggest that there is an intrinsic ability of the central circuitry to become entrained and that this ability increases with maturation.

The values of the stimulus phase in mature animals reported here (Fig. 4A) agree with those obtained from previous findings. Differences in the phase of the flight cycle at

which stretch receptor nerves are active result in differences in the length of the wingbeat period (25) and there is a preferred phase for entrainment, which is just after hindwing depressor activity (19).

A model of how the fSR can entrain and increase the wingbeat frequency in mature locusts has been presented (18). The authors suggest that there is a delay, mediated via interneuronal connections, between fSR activity and input of the fSR onto depressor motoneurons. The result is that fSR input occurs when the motoneuron is hyperpolarizing and thus the excitatory input reduces the extent of hyperpolarization. This allows the subsequent depolarization of the motoneuron to occur sooner, thus advancing the next wing depression (i.e., increasing the wingbeat frequency). In this study we show that the timing of depressor activity relative to fSR stimulation during co-ordination with an immature motor pattern is advanced relative to that of a mature motor pattern. Conversely, the timing of the stimulus within the depressor cycle during co-ordination is delayed in immature animals. Thus, according to the model [see (18)], fSR input onto a depressor motoneuron would be further delayed in immature animals, resulting in a less pronounced effect on the hyperpolarization of the motoneuron. Moreover, the variability of the stimulus phase observed during phase-locking of immature animals indicates a greater variability in the timing of the input onto the motoneuron. This would be expressed as a greater variability in the immature wingbeat frequency of intact animals. Indeed, the depressor frequency of intact, tethered immature locusts is more variable than that of mature, tethered locusts (data not shown). Unpublished observations suggest that an increase in the intensity of afferent input produces a greater synaptic response in a flight interneuron. Further investigations need to examine the effects of changing fSR input onto other motoneurons and interneurons as well. Moreover, stimulating all four stretch receptors (forewing and hindwing) could provide the flight system with the full complement of proprioceptive feedback due to wing elevation and may produce a tighter phase-locking during stimulus trials. It should be mentioned, however, that stimulating all four stretch receptors using the methods described here would require further dissection which would influence the operation of the motor pattern by damaging portions of the thoracic musculature. Moreover, it has been demonstrated that stimulating only the two forewing stretch receptors can produce effective entrainment (19).

We suggest that the changes described here demonstrate that the flight system of the locust changes more dramatically during maturation than previously assumed and that these changes are, in part, manifested via changes in the timing of proprioceptive feedback.

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