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Context dependency of a limb withdrawal reflex in the caterpillar *Manduca sexta*

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Abstract The proleg withdrawal reflex in the caterpillar *Manduca sexta* is a robust, well-characterized system for investigating the integration of sensory information with centrally patterned behavior. The reflex is evoked by stimulating mechanosensory hairs – planta hairs – located at the tip of each proleg. We studied the expression of this reflex by combining video recordings and electromyographic recordings from the main retractor muscles of the proleg, the principal and accessory planta retractor muscles. In intact animals, the nature of the response depended on the motor context of the animal. Animals which were standing quietly showed great variability in both the kinematic properties of proleg withdrawal, and the corresponding muscle electrical activity. Animals which were hanging upside down from a wooden dowel exhibited a much faster reflex, with retraction of the proleg occurring slightly faster than in standing animals, but re-extension of the proleg to the substrate being considerably faster. In crawling animals, expression of the reflex depended on the phase of the crawling cycle during which stimulation occurred. The reflex in a given proleg was suppressed during stance phase of that proleg. During swing phase, however, planta hair stimulation evoked proleg withdrawal, resulting in an assistance reflex. In contrast, isolated abdomens showed much less variability in the reflex. A comparison of the relationship between retractor muscle activity and the resulting proleg movement showed significant correlations between both the duration of activity and the number of muscle spikes, and the size of the associated proleg withdrawal. This is a promising system in which to investigate how central

neuronal circuits accomplish context-dependency of motor behavior.

Key words Insect · Locomotion · Motor control · Neuroethology

Abbreviations *APRM* accessory planta retractor muscle · *CPG* central pattern generator · *EMG* electromyogram · *EPSP* excitatory postsynaptic potential · *PPR* principal planta retractor neuron · *PPRM* principal planta retractor muscle

Introduction

One of the notable features of animal behavior is its “singleness” – motor systems rarely seem to be trying to do two things at once. How is this accomplished? The question has two subtle corollaries: (1) how is pertinent sensory information incorporated into centrally-generated motor patterns “to meet the exigencies of the environment” (Sherrington 1906)?, and (2) how is the potential conflict resolved when two behaviors, requiring the same motor apparatus, are triggered simultaneously? Insects offer promising model systems in which to investigate these issues, because many insect muscles are innervated by only one, or at most several, motor neurons. Since these neurons can be identified, circuits can be analyzed at the level of single cells, and a complete understanding of the mechanisms underlying behavioral modulation and coordination can be obtained.

Reflex modulation is a common feature of motor systems (see Prochazka 1989; Pearson 1995; Stein et al. 1997, for reviews). In both invertebrate and vertebrate systems, a single sensory input can have completely opposite effects, depending on when in the phasing of a particular behavior it occurs: so-called “reflex reversal”. For example, in both cats (Forssberg 1979) and locusts (Wolf 1992) stimulation of limb exteroceptors during walking can promote either flexion or extension, depending on whether the stimulus occurs during the swing or stance

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phase of the limb. The gain of reflexes may also be modulated either in different behaviors, or over the course of one phase of a particular behavior (e.g., the femoral chordotonal reflex in stick insects, Bässler 1976; the stretch reflex of knee extensor muscles in humans, Dietz et al. 1990). In many cases, a significant component of this modulation occurs via presynaptic modulation of transmission of primary afferents (in crayfish, El Manira et al. 1990; in cats, Gossard et al. 1989). A second, and equally important, form of regulation of reflex pathways is central modulation of interneurons in the circuits. Again, similar mechanisms have been observed in both vertebrates and invertebrates (stick insect, Büschges and Schmitz 1991; locust, Laurent and Burrows 1989; cat, Moschovakis et al. 1991; tadpole and others, Sillar 1991). The basis of this modulation in many systems is the existence of multiple, parallel pathways that can be differentially regulated. This occurs in di- and trisynaptic cutaneous pathways in the cat (Moschovakis et al. 1991), the walking system of the stick insect (Büschges and Schmitz 1991), and probably in humans as well (Duysens et al. 1991).

The proleg withdrawal reflex in caterpillars is an attractive system in which to study these issues. The reflex is initiated by stimulating mechanosensory hairs – planta hairs – located at the tip of each of the abdominal prolegs (Weeks and Jacobs 1987). The basic circuitry of the reflex is known (Fig. 1), and consists of mono- and polysynaptic connections between the mechanosensory afferents and identified motoneurons of the proleg retractor muscles, the principal planta retractor (PPRM) and the accessory planta retractor muscle (APRM) (Weeks and Jacobs 1987; Streichert and Weeks 1995; Sandstrom and Weeks 1996). The synapse between the afferents and the PPRM motoneuron (PPR) is particularly well characterized. The fast excitatory postsynaptic potentials (EPSPs) are mediated by nicotinic cholinergic receptors, but there are slower effects of muscarinic cholinergic receptors both pre- and postsynaptically that affect EPSP amplitude and motoneuron excitability (Weeks and Jacobs 1987; Trimmer and Weeks 1989, 1993; Trimmer 1994). In addition, there are a number of forms of activity-dependent plasticity: facilitation, depression and post-tetanic potentiation (Weeks and Jacobs 1987; Trimmer and Weeks 1991). While there are polysynaptic pathways involved in the reflex, both intra- and interganglionic, these remain largely unidentified (Weeks and Jacobs 1987; Wiel 1995; Sandstrom and Weeks 1996; Wiel and Weeks 1996). The proleg withdrawal reflex habituates in response to repeated planta hair stimulation, and dishabituates following strong or noxious stimuli (Wiel and Weeks 1996; Wood et al. 1997). While there is some decrement in sensory transmission during habituation, there is little change in the direct afferent to PPR synapse, and the primary locus of habituation appears to be in the polysynaptic pathway(s) (Wiel 1995). Hence, this system displays modulatory properties common to many reflex pathways, with most of its components being particularly well described, and it is amenable to study using reduced preparations.

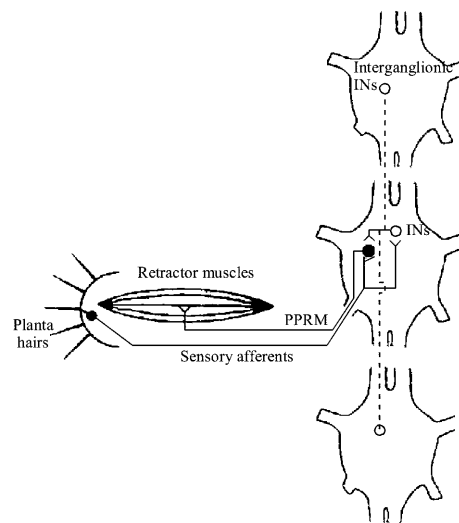


Fig. 1 Schematic diagram of the components of the proleg withdrawal reflex. The interganglionic pathways are inferred from experiments showing that the number of attached segmental ganglia affects the strength of the response (Wiel 1995). *INs*, interneurons. Adapted from Wiel and Weeks (1996)

We used this well-characterized system to examine the integration of reflex responses with other behaviors, as a prelude to examining the central circuitry underlying these changes. While there have been anecdotal reports of context-dependency for this reflex (Weeks and Jacobs 1987), no systematic investigation has been performed. Also, there are no data on the relationships between activity of the retractor muscles and behavioral responses during reflex activation. We have recently examined the relationships between retractor motoneuron activity and proleg movement during crawling (Belanger and Trimmer 2000), and the present study extends this work to include reflex activation. We videotaped animals while simultaneously recording the electrical activity of PPRM and APRM. We found that the proleg withdrawal reflex was strongly modulated by the behavioral context of the animal. The reflex was quite variable in intact, quiescent animals. In animals which were hanging upside down, the reflex was considerably faster, and it was suppressed during the stance phase of crawling. During the swing phase of crawling, the reflex was present, acting as an assistance reflex. Isolated abdomens showed much less variability in the reflex. In contrast to retractor muscle activation during crawling, there were significant correlations between muscle activation, as measured using electromyograms (EMGs), and the extent of proleg movements. Portions of this work have appeared previously in abstract form (Belanger et al. 1999).

Materials and methods

The animals and methods used in this paper are presented in detail in Belanger and Trimmer (2000). Briefly, electromyographic electrodes were implanted into the muscles of interest, and animals

were videotaped as they behaved on wooden dowels. Synchronization pulses present on both the EMG data records and the videotape allowed subsequent correlation of proleg movements with underlying muscle activity. For experiments on isolated abdomens, animals were anesthetized on ice for at least 30 min. They were then ligatured with silk thread at the juncture of the thorax and abdomen, and the anterior portion of the animal removed. The resulting preparation was maintained at 4 °C for 24 h. Electrodes were then implanted for EMG, and the preparation left to recover at 22 °C for 1 h before experimentation.

A small brush was used to deflect the planta hairs to evoke the proleg withdrawal reflex. In each case, the planta hairs were brushed in the anterior to posterior direction, and the stroke was aimed to contact as many of the planta hairs as possible. Contact of the brush with the hairs was always confirmed on the videotape by ensuring that either the planta hairs or the brush bristles could be seen to move. Based on the video records, the stimuli lasted approximately 60–150 ms. Informal testing using a piezoelectric crystal suggested that the variability in the force of the stimulus across trials was less than 15%, probably due to the compliance of the brush bristles. To avoid effects due to habituation (Wiel 1995; Wiel and Weeks 1996; Wood et al. 1997), tactile stimuli were delivered at least 5 min apart.

The EMG and kinematic data reported here for intact animals consist of at least four evoked reflexes from each of ten animals under each set of conditions. Averages are given as mean \pm standard deviation (SD). A critical level of $P < 0.05$ and two-tailed probabilities were used throughout this study. Comparisons between two or more groups used ANOVA (for repeated measures where appropriate), followed by pair-wise comparisons using the Newman-Kewels post-hoc test where appropriate (Sokal and Rohlf 1995). Ratio variables were transformed using square root transformations prior to testing (Sokal and Rohlf 1995).

Results

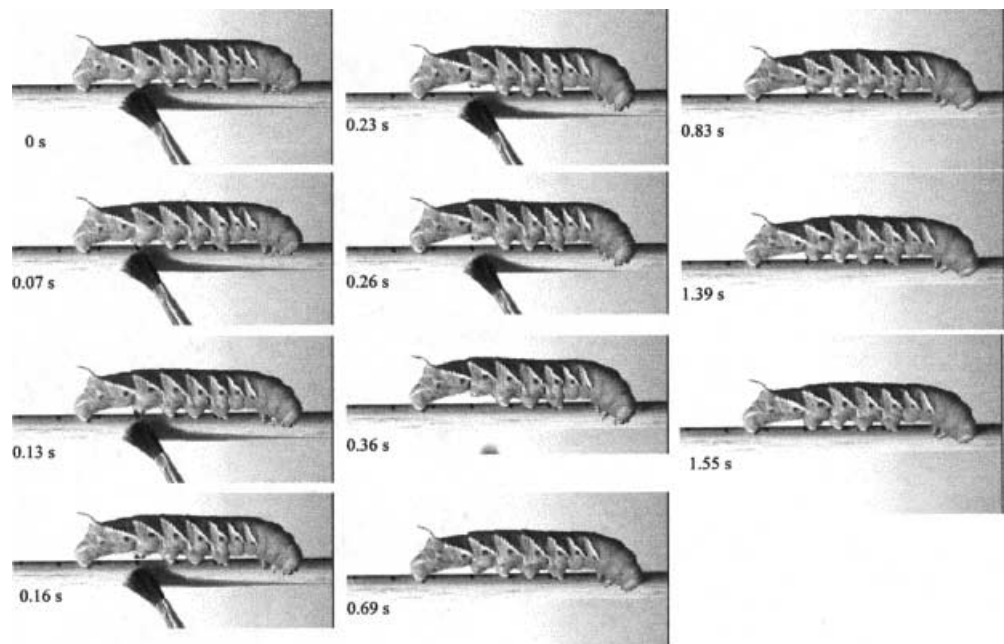
The proleg withdrawal reflex could be elicited reliably in intact, quiescent, animals by stroking the planta hairs with a small brush (Fig. 2). As has been noted previously (Weeks and Jacobs 1987), there was considerable

variability in both the magnitude and duration of the response. In general, the response consisted of activation of both PPRM and APRM, resulting in the planta being withdrawn into the proleg, and the proleg tip retracted towards the body. There was usually also contraction of longitudinal muscles in the stimulated segment, so that the animal seemed to “flinch”. (We did not study this aspect of the reflex further.) When the animal was standing right-side up, the initial retraction of the proleg was much faster than the subsequent re-extension (Fig. 3A). This is probably due to the fact that, while there are retractor muscles for the prolegs, there are no extensor muscles. Instead, they must be extended by hydrostatic pressure.

Caterpillars often hang upside down from their perches during normal behavior, so we examined the expression of the proleg withdrawal response in this condition. The reflex could still be elicited by stroking the planta hairs, but the resulting retraction was of much shorter duration (Fig. 3B). Both the actual retraction of the proleg, and the underlying muscle activity, were only slightly smaller than in the right-side up condition, but re-extension of the proleg was much faster.

Since the prolegs are used by the animal to grasp the substrate when crawling, there is a potential conflict between crawling and proleg withdrawal if the planta hairs are stimulated during this behavior. To investigate the resolution of this conflict, we stimulated the planta hairs in animals that were crawling. Because *Manduca* crawl fairly slowly (the cycle period is approximately 1–3 s on the dowels we used), it was possible to stimulate the planta hairs at various phases during the step cycle of a proleg. When the planta hairs of a proleg that was in stance phase (contacting the substrate and supporting the animal) were deflected, there was generally no

Fig. 2 Example of a proleg withdrawal reflex in an intact animal to show the kinematics of the proleg. At 0 s the brush contacts the planta hairs of the proleg on abdominal segment 6. From 0 s to 0.23 s the proleg retracts. At 0.36 s the proleg begins to re-extend, the crochets become visible at 1.39 s, and at 1.55 s the proleg regains contact with the substrate



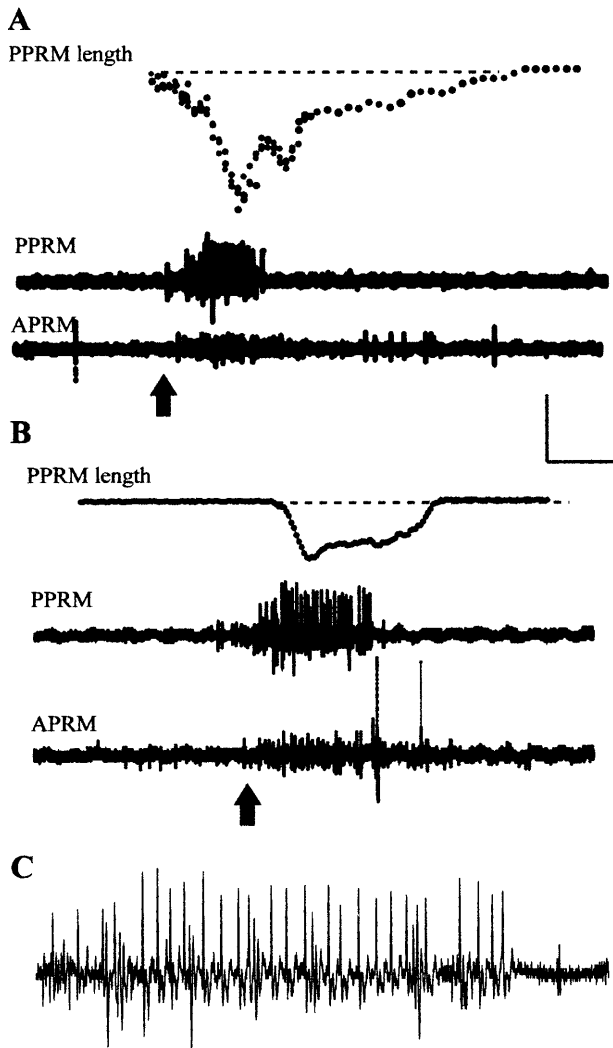


Fig. 3 Typical proleg withdrawal reflexes invoked in animals standing right-side up (**A**) or upside down (**B**) on a wooden dowel. The *top trace* in each case shows the change in length of principal planta retractor muscle (PPRM), based on the measured distance from the muscle's origin to insertion. (Note that the muscle length in this and all other figures is an approximation based on the measured distance from the muscle's origin to its insertion.) The *lower two traces* show electromyogram (EMG) responses measured from PPRM and accessory planta retractor muscle (APRM). The approximate start of the stimulus is indicated by the *arrows*. **C** The same PPRM burst as in **B** at an expanded timescale to show individual muscle spikes. Scale bars in **A**, **B**: 2 mm, 100 μ V, 1 s; in **C**: 50 μ V, 0.27 s

retraction of the proleg, and only occasionally a slight burst of activity in the retractor muscles (Fig. 4A). (We also noted that there was a general suppression of the reflex when the animal had reared up and was waving its head and thorax in the air, but we did not investigate this in detail.) In contrast, stimulating the planta hairs during the swing phase of crawling evoked a reflexive response (Fig. 4B). The retractor muscles are used during crawling to disengage the hook-like crochets at the proleg tip from the substrate (Belanger and Trimmer 2000). Therefore, to detect an effect of planta hair

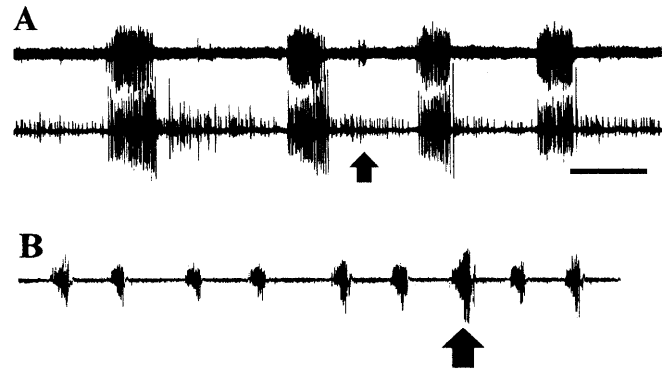


Fig. 4A, B Effects of planta hair stimulation during crawling. Animals were allowed to crawl on a wooden dowel, and the planta hairs were stimulated with a small brush. **A** When the animal is crawling, the bursts of activity associated with proleg swing are clearly visible in the traces from PPRM (*top*) and APRM (*bottom*). Stimulating the planta hairs (indicated by *arrow*) when the proleg is in stance phase produces no visible movement of the proleg, and little activity in the EMG records. **B** In contrast, stimulating the hairs when the proleg is in swing phase evokes a proleg withdrawal reflex, as seen by the increased EMG activity. (Only the PPRM trace is shown.) See Fig. 5 for kinematic data. *Arrows* mark the approximate onset of the stimulus. Scale bars in **A**: 1 s; in **B**: 2 s

stimulation, we compared proleg kinematics and muscle activity in perturbed steps with normal steps during a given crawling sequence (Fig. 5). During a normal step, retractor muscle activity only returns the proleg, which has been stretched by abdominal contractions, to its normal length (Fig. 5; see Belanger and Trimmer 2000). Planta hair stimulation, however, altered the kinematics and muscle activity of the affected proleg. There was a slight tendency for perturbed steps to have a greater stride length (5% greater, on average, than control steps), but the difference was not significant. There was also a slight, but not significant, decrease in period of the step cycle (1.8 ± 0.4 s versus 2.0 ± 0.7 s, $P = 0.078$), and in the relative duration of swing phase in the step (0.29 ± 0.09 versus 0.32 ± 0.08 , $P = 0.74$). There was, however, a significant increase in both the absolute duration of activity of PPRM (0.83 ± 0.28 s versus 0.51 ± 0.14 s, $P = 0.01$), and when this was expressed as a proportion of the cycle period (0.39 ± 0.23 versus 0.27 ± 0.15 , $P = 0.01$). As a result of the longer bursts, perturbed steps had greater numbers of PPRM spikes per step (27 ± 7 versus 22 ± 3 , $P = 0.05$), and they also had a slightly higher maximum frequency (70 ± 15 versus 61 ± 14 Hz, $P = 0.05$). This combination produced greater shortening of the muscle (to 77 ± 0.04 versus $83 \pm 0.03\%$ of rest length, $P = 0.007$), and a longer period of retraction before re-extension. The result of these factors was that the proleg was carried higher during a perturbed step, although its overall motion was still governed largely by the abdominal movements (see Belanger and Trimmer 2000).

In order to make meaningful comparisons with the reduced preparations we use for intracellular recordings, we also examined the reflex in isolated abdomens. These preparations will exhibit righting reflexes, but generally

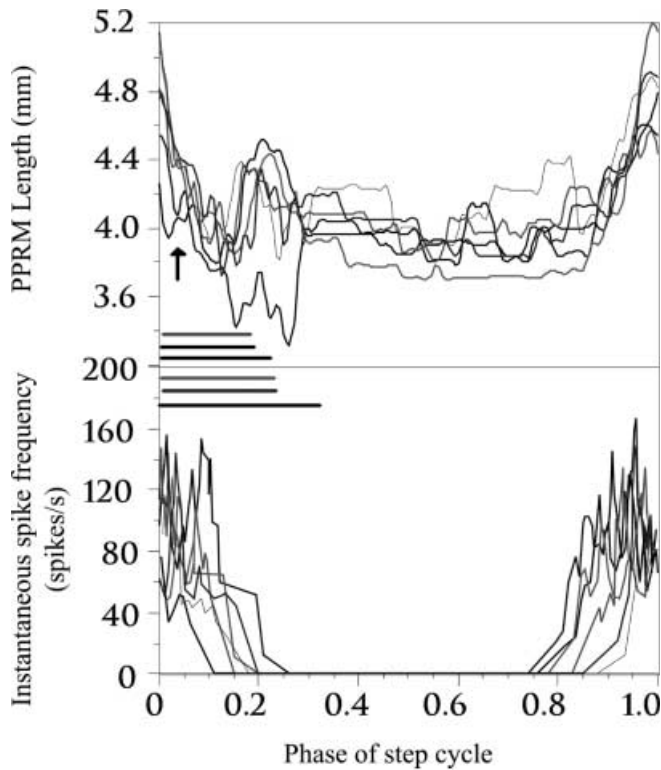


Fig. 5 The effect of stimulating planta hairs during the swing phase of a proleg when the animal is crawling. The *upper panel* shows the length of PPRM during a step, normalized to the step cycle duration, with 0 phase defined as the onset of swing. The *lower panel* shows the instantaneous spike frequency of PPRM, measured by binning spikes into 50-ms periods. Six consecutive steps are shown: five control steps (*thin lines*) and one perturbed step (*thick lines*, planta hair stimulus applied at *arrow*). The *solid lines* between the traces mark the duration of swing for each of the steps. Note that PPRM contracts to a greater degree, for a longer period, when the planta hairs are stimulated. There is a concomitant increase in the frequency of spikes and duration of activity in PPRM

do not crawl, and will not hang upside down from dowels. Nonetheless, we could elicit the reflex in standing animals, and make comparisons with the equivalent condition in intact animals (Fig. 6). The major difference is that there is considerably less variability in the reflex in isolated animals, as noted previously by Weeks and Jacobs (1987).

When these behaviors were averaged across animals and compared, a clear effect of behavioral context emerged (Fig. 7). All aspects of the reflex were effectively suppressed during the stance phase of crawling. The total duration of the reflex was less in animals which were hanging upside down than were standing (1.8 ± 1.7 versus 3.2 ± 2.9 s, $P < 0.01$), but the retraction portions of the reflex (muscle activity, shortening of the muscles) were only slightly reduced. Most of the decrease in total duration of withdrawal was due to faster re-extension of the limb. The duration of the reflex was also significantly shorter in isolated abdomens, with an average proleg retraction of 1.5 ± 0.7 s. Interestingly, the variability in the reflex was much less in the isolated abdomens

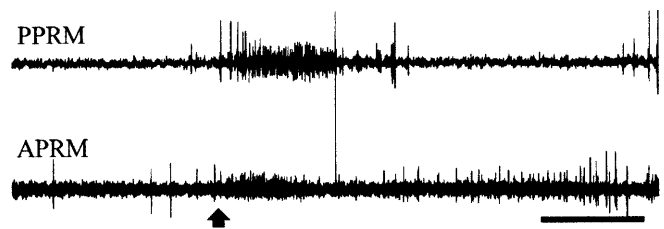


Fig. 6 Effects of planta hair stimulation in an isolated abdomen. The preparation was "standing" on a flat substrate, and planta hairs were stroked with a brush at the *arrow*. There is a burst of activity in PPRM (*top trace*), and a much smaller burst of activity in APRM (*lower trace*). Scale bar: 0.5 s

(coefficient of variation of 47% for the isolated abdomens, versus 91% and 94% for standing and dangling animals, respectively).

In a previous investigation of the activity of the proleg retractor muscles during crawling, we found that there was only a weak correlation between the degree of muscle activation and the actual shortening of the proleg (Belanger and Trimmer 2000). We hypothesized that this was because the retractor muscles are operating against the hydrostatic pressure generated by the animal during crawling. Since their major role is to disengage the crochets in preparation for the swing phase of a step, there is no adaptive pressure to match kinematic output with muscle input. To determine if this was a general property of the retractor muscles, or was specific to crawling behavior, for PPRM we regressed the duration of muscle bursts, or the total number of muscle spikes, against the resulting amount of shortening of the muscle. (Since PPRM is innervated by only one motoneuron, the timing of muscle spikes is an accurate reflection of the activity of the motoneuron.) There was a significant correlation between both of these variables and the resulting degree of shortening by the muscle (Fig. 8A, B). The coefficients of determination, representing the amount of variability in muscle contraction which was accounted for by the variables, were 75% for burst duration and 72% for number of muscle spikes.

In contrast, the correlations between properties of muscle electrical activity and the resulting muscle contraction in isolated abdomens were much weaker (Fig. 8C, D). The only significant correlation was with burst length, and the coefficient of determination was only 27%.

Discussion

Using combined kinematic and electromyographic analysis, we have shown that a physiologically well-characterized motor reflex, the proleg withdrawal response in the caterpillar *M. sexta*, shows a strong dependence on behavioral context. These modulations of the reflex have clear adaptive value. In some contexts, the reflex is an avoidance response, similar to that involving exteroceptors in other insects (e.g., Pflüger

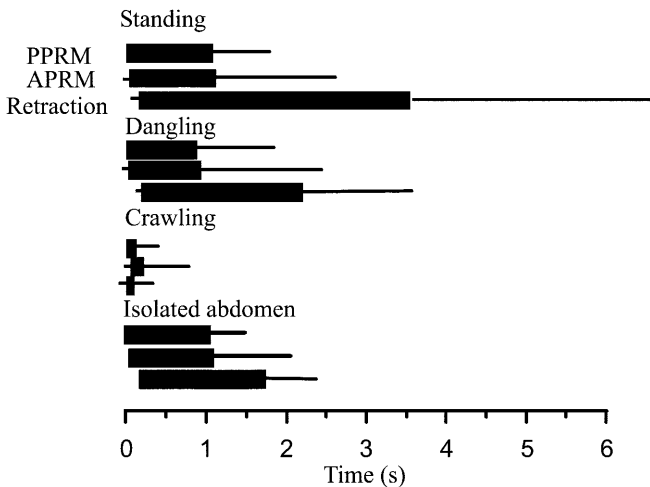


Fig. 7 Averaged differences in kinematics and EMG responses of the withdrawal reflex in intact animals and in isolated abdomens. In each case, the reflex was initiated by stimulating a group of planta hairs, including the α -hair, with a small brush. *Standing*: averaged properties of the reflex evoked in animals standing on a dowel. The *top two bars* show averaged EMG activity of PPRM and APRM. The *start of the bar* represents onset time relative to PPRM (error bars give standard deviation of onset). The *length of a bar* represents the duration of activity, with error bars for standard deviation of duration. The *lower bar* represents time spent by the proleg raised from the substrate. *Dangling*: averaged properties of the reflex evoked in animals hanging upside down from the dowel. *Crawling*: averaged properties of the reflex evoked in animals during the swing phase of crawling. The *bars* show the contribution to a step made by reflex activation, obtained by subtracting the values for control (non-perturbed) steps from stimulated steps. *Isolated abdomen*: averaged values from isolated abdomens standing on a flat surface

1980). The decreased duration of the reflex in upside down animals would obviously help prevent the animal from falling from the substrate, which is also likely to be its food supply. Suppression of the reflex during the stance phase of crawling probably serves the same aim. (It also avoids the difficulty of the animal producing a withdrawal response every time it tries to put its 'foot' down.) During the swing phase, however, the withdrawal response functions as an assistance reflex, analogous to that seen in many walking animals (Forsberg 1979; Wolf 1992). In these cases, contact of the planta hairs is presumably interpreted by the animal as an obstacle, and an increased retraction of the leg may be necessary to clear the obstacle.

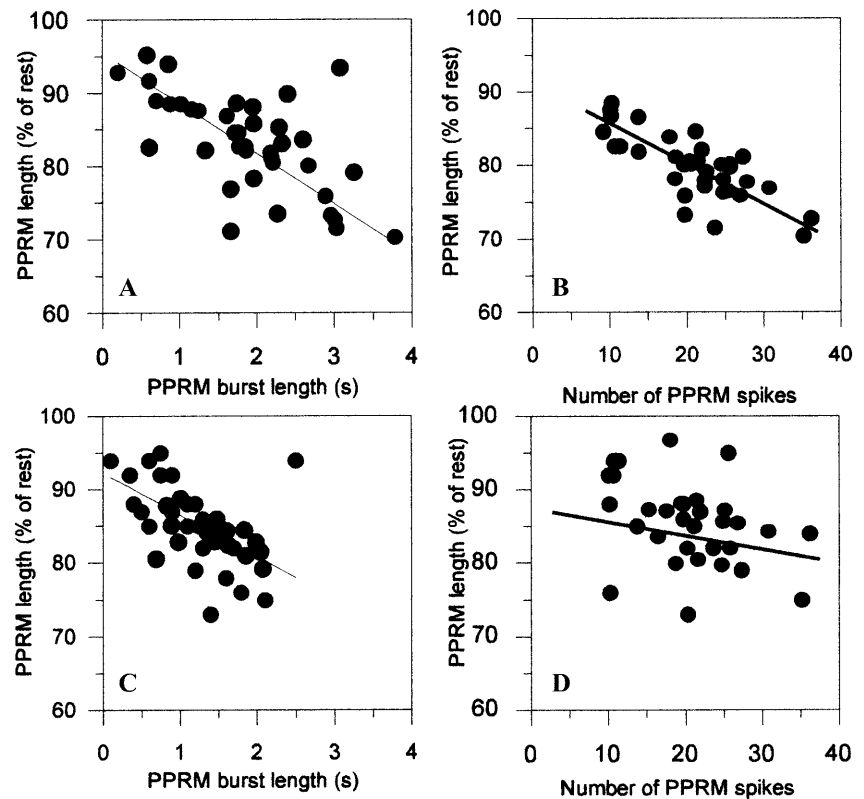
These results do not allow us to identify the locus (or loci) of modulation. Based on other systems, it is probable that there are several. Increased activity of the planta hair afferents activates presynaptic muscarinic acetylcholine receptors, leading to a down-regulation of transmitter release, and, hence, a down-modulation of the activity of this pathway (Trimmer and Weeks 1989). The planta hairs contact the ground at every step during crawling, and so could be expected to undergo this down-regulation. While the precise mechanism of the decreased transmitter release is not known, a likely

mechanism is primary afferent depolarization (PAD). PAD is seen in many other systems (Watson 1992), and affords the central nervous system a way to tailor the gain of sensory inputs to match the current behavioral needs of the animal. For instance, Burrows (1996) has argued that the presynaptic depolarization of sensory terminals that occurs during walking in the locust acts to reduce the effect of sensory inputs that are a result of self-induced motion.

A second source of down-regulation of the reflex could be habituation. Experimentally, habituation can be induced with interstimulus intervals that are considerably greater than the stepping period during crawling (Wiel 1995; Wiel and Weeks 1996; Wood et al. 1997). However, these sources of decreased sensory input cannot explain all of the modulation of the proleg withdrawal response. Since the response can be elicited during the swing phase of crawling, sensory inputs are still present. A possible mechanism for maintaining the motor role of the reflex in the face of these decreases in sensory input also involves muscarinic receptors. At least PPR, and probably all of the proleg motoneurons, possess muscarinic acetylcholine receptors which increase the excitability of the neurons (Trimmer 1994). Muscarinic inputs, then, appear to play a key role in balancing the down-regulation of sensory inputs with up-regulation of motor responses. In the crayfish, octopamine plays a similar role of modulating reflexes in a steady-state manner (Skorupski 1996). It is interesting to note that both of these "steady-state reflex modulators" can induce the expression of fictive behaviors in isolated ganglia: the muscarinic agonist pilocarpine initiates fictive walking in the locust (Ryckebusch and Laurent 1993), and crawling in *Manduca* (Johnston and Levine 1996), while octopamine initiates fictive flight in the locust (Stevenson and Kutsch 1988).

These steady-state effects may explain some of the modulatory effects we have seen, particularly the lack of modulation and relative stereotypy seen in isolated abdomens. It is clear, however, that the phasic modulation of the reflex seen during crawling must occur on a faster timescale. Laurent and Burrows (1989) have demonstrated in the locust how intersegmental interneurons can play this role via their actions on non-spiking local interneurons. While non-spiking interneurons have not been demonstrated in *Manduca* (or any other lepidopteran), it seems probable that they exist, and, if not, that spiking interneurons will be found that play a similar role. A major advantage of this system is that we know where to look for such interneurons. Since fictive crawling can be induced in isolated ganglia by pilocarpine (Johnston and Levine 1996), it is now possible to look for inputs to motor and interneurons that modulate their responsiveness in phase with the crawling rhythm. However, the present work makes clear that it will be essential to deliver stimuli during the appropriate phase of activity in order to determine the behavioral relevance of central connections.

Fig. 8 Correlations between PPRM burst properties and movement of the proleg in standing animals (**A, B**) and isolated abdomens (**C, D**).
A $r^2=0.75$, $\beta=-6.9$, $P<0.001$;
B $r^2=0.72$, $\beta=-0.58$, $P<0.001$;
C $r^2=0.27$, $\beta=-5.7$, $P=0.009$;
D $r^2=0.07$, $\beta=-0.18$, $P=0.14$



It is worth noting that the major significant effect of planta hair stimulation during the swing phase of crawling animals was on the duration of muscle activity. Since there were only small changes in the number and frequency of muscle spikes, this suggests that input to the motoneuron from the reflex pathway is not being simply added to that from the central pattern generator (CPG) which is thought to drive crawling behavior (Johnston and Levine 1996). That is, reflex activation is not merely being overlaid onto the crawling rhythm, but rather is being incorporated into the ongoing rhythmic activity. This idea is supported by the lack of significant increases in the duration of swing, and therefore stride length. It probably reflects the tight coupling between the phasing of stepping and the activity of the retractor muscles, a constraint of the fact that crawling behavior is driven mainly by abdominal muscles, with the prolegs acting as claspers, and the retractors' main role being to operate the crochets (Belanger and Trimmer 2000).

Despite the lack of a significant increase in the number of muscle spikes in response to sensory stimulation, there was a significant correlation overall between the number of spikes and the resulting muscle contraction. This is in direct contrast to our earlier finding that, in normally crawling animals, there is no strong relationship between the intensity of the motoneuronal input and the degree of muscle contraction (Belanger and Trimmer 2000). One possible explanation lies in the observation that, during normal crawling, the retractor muscles are opposing the increased hydrostatic pressure resulting from the abdominal contractions which provide most of

the motive force. The increased duration of muscle activity in response to reflex activation may allow the muscle to operate after the hydrostatic pressure has reduced, unmasking a previously obscured relationship. (Similar variation in the hydrostatic pressure in the isolated abdomens may explain the lack of significant correlation in these preparations.) A second, quite likely, possibility is that no single, simple measure of motoneuronal input is sufficient to predict muscle activation; instead, the complex pattern of activity throughout the motoneuronal burst encodes the commanded movement.

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