Jim H. Belanger · Kevin J. Bender · Barry A. Trimmer

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

Received: 29 September 2000 / Published online: 11 November 2000 © Springer-Verlag 2000

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...
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 (Büsches and Schmitz 1991), and probably in humans as well (Duyens 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 (PR) is particularly well characterized. The fast excitatory postsynaptic potentials (EPSPs) are mediated by nictotinic 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](image)

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