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SHORT COMMUNICATION

REFLEX PATHWAYS RESPONSIVE TO DEPRESSION OF THE LOCUST COXOTROCHANTERAL JOINT

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Resistance reflexes in arthropods are negative feedback reflexes broadly analogous to the vertebrate stretch reflex (Bush, 1962). Such reflexes may well be concerned with the maintenance of stable posture as a prerequisite for motor behaviour. Proprioceptors in the arthropod limb monitoring position and movement include muscle receptor organs and the chordotonal organs (see Mill, 1976, for reviews). In insects, an additional class of joint receptor has been described: the strand receptors (Bräunig and Hustert, 1980; Bräunig *et al.* 1981; Bräunig, 1982). The cell bodies of strand receptor primary afferent neurones are located within the segmental ganglia rather than at the peripheral sense organ.

The coxotrochanteral joint of the locust is equipped with two strand receptors (SRs) and one muscle receptor organ (MRO), which all respond to depression of the joint. Selective stimulation of each of these receptors can generate a resistance reflex in trochanteral levator motoneurones (Bräunig and Hustert, 1983). In arthropods, local proprioceptive reflexes are mediated by direct connections from afferent neurones to motoneurones (Blight and Llinas, 1980; Burrows, 1975, 1987*a*; Pearson *et al.* 1976; Skorupski, 1991). In addition, in the locust, both tactile and proprioceptive afferents are mapped onto a layer of spiking local interneurones, that can, in turn, influence motoneurones directly and indirectly through a layer of nonspiking local interneurones (Burrows, 1987*a*,*b*; Burrows *et al.* 1988; Laurent and Burrows, 1989; Siegler and Burrows, 1983). Motoneurones thus receive both mono- and polysynaptic primary afferent input. This scheme derives largely from studies on distal afferents, but less is known about the reflex organization of the proximal leg joints. The morphology, innervation and reflex actions of coxal proprioceptors are documented (Bräunig, 1982; Bräunig *et al.* 1981; Bräunig and Hustert, 1983), but their central synaptic pathways are not yet known. In this paper we present evidence that coxotrochanteral strand receptor

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afferents make direct excitatory connections with levator motoneurones, and that these connections underlie, at least in part, the resistance reflex evoked by depression of the coxotrochanteral joint.

Experiments were carried out on adult locusts, *Schistocerca gregaria* (Forskål), taken from our crowded laboratory culture 1–2 weeks after their final moult. Locusts were restrained, ventral surface uppermost, in plasticene and the coxa of the left hindleg was fixed at right angles to the thorax with superglue. The trochanter (and femur with which it is fused) were levated by about 30° relative to the longitudinal axis of the coxa. From this resting angle, depressions of about 30° were imposed manually by gently lifting the femur. In some experiments, nerve 5B was severed near the ganglion to exclude any tactile input from this method of stimulation.

Nerve 3B5, which carries the afferent innervation of the coxotrochanteral SRs 1 and 2 and the coxotrochanteral MRO, was exposed in the coxa after removing the trochanteral depressor muscle 133a and the levator muscles 131a and b. Monopolar recordings were made from nerve 3B5 using a fine insect pin, bent at the very tip. Nerve 3B was usually cut distal to the emergence of 3B5, thereby removing any remaining motor innervation of levator muscle 131 (Bräunig, 1982). Nerve 3B was also recorded *en passant*, within the thoracic cavity, using bipolar hook electrodes. Nerves 3C and 5A were cut to denervate trochanteral depressor muscles and the coxal hairplates and rows of hairs (Bräunig, 1982). Nerve 4A (which innervates levator muscles 132a and 132b) was cut and drawn into a suction electrode for extracellular recording from the proximal stump.

The meso- and metathoracic ganglia were stabilized on a wax-coated platform and treated with a 1 % (w/v) solution of protease (Sigma type XIV). Intracellular recordings were made with microelectrodes filled with 2 mol l^{-1} potassium acetate (d.c. resistances of about 50 MΩ) or, sometimes, 6 % cobalt hexammine chloride. Impaled levator motoneurone somata were identified by an axon in nerve 4A or 3B. Impaled somata of SR afferents were identified by stimulating the peripheral nerve (3B5) and, in some cases, by intracellular staining and silver intensification after ionophoresis of cobalt ions from the electrode.

All data were stored on FM tape. Subsequently, recordings were digitized using a CED 1401 laboratory interface and microcomputer, and analysed using commercial software (SIGAVG, SPIKE2: CED). For spike-triggered averaging, extra- and intracellular recordings were digitized at 10 kHz. Cross-correlograms (Cope *et al.* 1987) were constructed using pulses derived from extracellularly recorded spikes. A segment of data taken between 3 and 10 ms before the occurrence of the trigger spikes and 15–25 ms after them was divided into 0.1 ms bins. Variations in the probability of occurrence of a test spike could then be determined relative to the time of the trigger spike. Spikes for triggering signal averaging and cross-correlation were discriminated on the basis of both height and duration using a CED 1401–18 multichannel window discriminator.

Depression of the coxotrochanteral joint invariably evoked a resistance reflex in which a number of levator motoneurones were driven to spike (Fig. 1). Typically

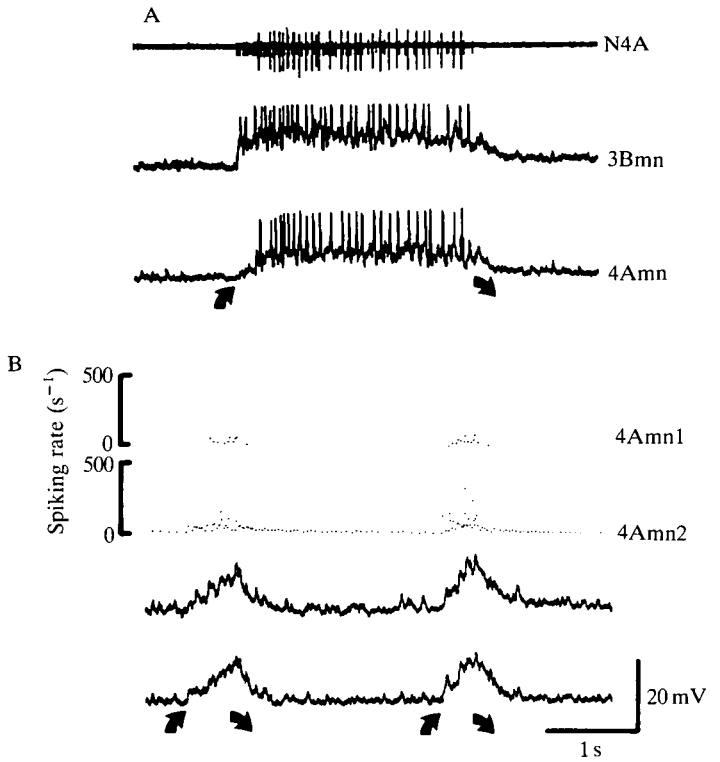

Fig. 1. Resistance reflexes evoked by depression of the coxotrochanteral joint. (A) Simultaneous recordings from nerve 4A (upper trace) and intracellularly from two levator motoneurones (mn), one with an axon in nerve 4A (third trace; note 1:1 correlation of intracellular spikes with large spikes in the extracellular recording) and the other with an axon in nerve 3B (second trace; extracellular recording from nerve 3B not displayed). Upward arrows indicate approximate timing of manually imposed depression of the coxotrochanteral joint; at the downward arrows the joint was returned to its resting position. (B) Subthreshold input to two levator motoneurones during joint depression (third and fourth traces). The coxotrochanteral resistance reflex is monitored as the instantaneous spiking frequency of two other units recorded extracellularly from nerve 4A.

in nerve 4A a single unit was tonically active and was excited further by joint depression, while a larger, previously silent, unit was also recruited (Fig. 1B). The activity in nerve 3B was somewhat more variable, but two or more levator units could always be activated by depression. Intracellular recording revealed that many levator motoneurones did not spike in response to depression, but such motoneurones nevertheless received subthreshold excitation (Fig. 1B).

In simultaneous recordings from pairs of levator motoneurones, patterns of apparently common synaptic input were observed (Fig. 1B). As with most other insect motoneurones (Burrows, 1978; Fourtner and Pearson, 1977), no evidence was obtained for electrical or chemical synaptic coupling. Injection of depolarizing

current into one neurone never produced any potential changes in the other, or any modulation of the spiking activity of other levator units recorded extracellularly from nerves 3B or 4A.

In 12 experiments multi-unit recordings of afferent activity were obtained from nerve 3B5, which contains the axons of the two coxotrochanteral strand receptors and that of the single multipolar sensillum of the coxotrochanteral MRO (Bräunig, 1982). Discrete EPSPs following spikes in nerve 3B5 were seen in 12 levator motoneurones (Fig. 2A), and spike-triggered averaging revealed an EPSP occurring 5.6–7.4 ms after an afferent spike (Fig. 2B). This delay includes the conduction time for the afferent spike to travel the 10–15 mm from the peripheral recording site on nerve 3B5 and at least one central synaptic delay.

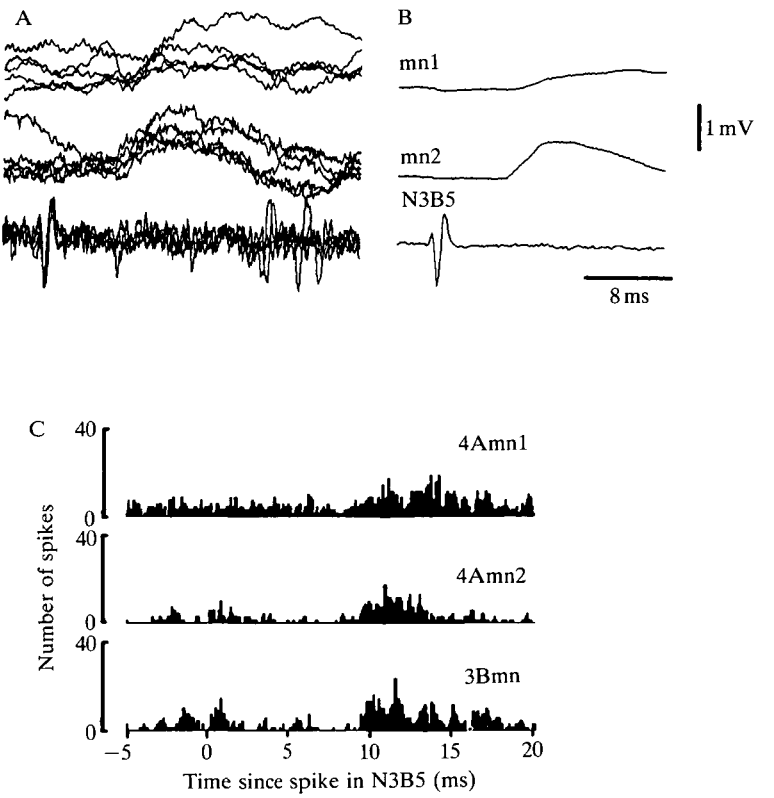

Fig. 2. Input from nerve 3B5 afferents to levator motoneurones. (A) Superimposed sweeps triggered from sensory spikes (third trace, N3B5) reveal constant-latency synaptic potentials in two levator motoneurones recorded intracellularly (first and second traces, mn1 and mn2). (B) Averaged recordings triggered from 48 sensory spikes. Traces as in A. (C) Cross-correlogram for two motoneurones recorded extracellularly from nerve 4A (first and second traces, 4Amn1 and 4Amn2) and a single motoneurone from nerve 3B (third trace, 3Bmn), triggered from sensory spikes in nerve 3B5. The peak probability of a motor spike follows the occurrence of afferent spikes by 8–10 ms.

The amplitude of single afferent EPSPs revealed by signal averaging ranged from 0.2 to 1.0 mV, which is fairly small compared to the net depolarization of 6–8 mV evoked in a motoneurone by depression of the coxotrochanteral joint (the maximum number of afferents contributing to this net depolarization is 8 or 10; Bräunig, 1982). Cross-correlation analysis, however, suggests that single afferents can be effective in modulating motoneuronal activity. Cross-correlation histograms of the occurrence of levator motoneurone spikes in nerves 4A or 3B (triggered from the same afferent spikes used for signal averaging of intracellularly recorded EPSPs) display peaks with 8–10 ms delay (Fig. 2C). This somewhat increased delay compared to signal-averaged EPSPs presumably reflects the additional time required for conduction of efferent motor spikes from the metathoracic ganglion to the peripheral nerve recording site (some 3–4 mm), as well as a central integration time in the motoneurone.

In the absence of precise measurements of afferent and efferent conduction velocity it cannot be stated whether the connections between coxotrochanteral afferents sensitive to joint depression and levator motoneurones are direct. To resolve this question, intracellular recordings were made simultaneously from the central cell bodies of SR afferents and levator motoneurones. The somata of SR afferents lie in a cluster immediately adjacent to the soma of the slow extensor tibiae motoneurone (SETi). Penetration of an SR afferent was confirmed by movement of the coxotrochanteral joint and stimulation of nerve 3B5. Orthodromic spikes followed the stimulation at constant latency and without prepotentials. In a few cases, stimulation of nerve 3B alone was used for identification, but SR afferents could readily be distinguished from other neurones that have their axons in this nerve (levator motoneurones and the SETi). Stimulation at intensities below or well above that required for generating spikes failed to generate any synaptic potentials (Fig. 3A) in the SR afferent. This contrasts with levator motoneurones, in which nerve 3B stimulation invariably evoked a compound EPSP as well as (in the case of motoneurones with 3B axons) an antidromic spike.

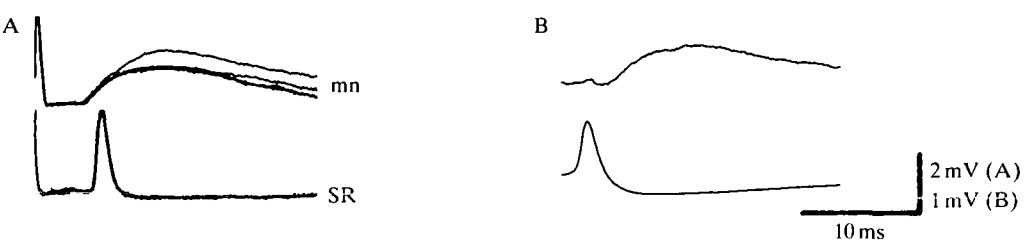

Fig. 3. Simultaneous intracellular recordings from a levator motoneurone and the central cell body of a strand receptor afferent. (A) Stimulation of nerve 3B evokes a compond EPSP in the motoneurone and an orthodromic spike in the afferent. (B) Signal averaging triggered from afferent spikes reveals an EPSP following in the motoneurone at a latency of about 1.3 ms.

The SETi could easily be identified and excluded by criteria described elsewhere (Heitler and Burrows, 1977). Two of the neurones identified as SR afferents were stained by ionophoresis of cobalt ions from the microelectrode. This revealed neurones with characteristic SR morphology (Bräunig and Hustert, 1980; Bräunig, 1982; Bräunig *et al.* 1981), thus confirming the physiological identification. Simultaneous intracellular recordings were obtained from an SR afferent and a levator motoneurone on five occasions. On four of these, triggering a signal averager from the afferent spikes revealed an EPSP in the motoneurone at a latency of 1.3–1.9 ms (Fig. 3B).

Depression of the locust coxotrochanteral joint evokes a resistance reflex involving a number of motoneurones (Fig. 1). All intracellular recordings from levator motoneurones revealed a similar pattern of excitatory inputs, although these remained subthreshold in many motoneurones. This input must result from divergent afferent connections, rather than lateral coupling in the motoneurone pool, since no evidence was detected for any form of coupling between levator motoneurones. This (with some well-known exceptions: Heitler and Burrows, 1977), appears to be an organizational principle of insect (as well as mammalian) motor systems. If so, then it is in stark contrast to an equivalent one in decapod crustaceans, where central coupling of motoneurones appears to be the rule (Nagayama *et al.* 1983; Simmers and Bush, 1983; Heitler, 1978; Skorupski and Sillar, 1988). In the crayfish, direct evidence has been obtained for the importance of lateral excitatory and inhibitory interactions within and between motoneurone pools in reflex integration (Skorupski and Sillar, 1988).

There are three possible receptors that could account for the observed input to levator motoneurones: the two strand receptors and the single MRO of the coxotrochanteral joint. SR2 and the MRO are each innervated by a single sensory neurone and SR1 is innervated by up to 10 sensory neurones. All of our recordings from nerve 3B5 displayed multiunit activity (Fig. 2) and we could not determine which, if any, of the afferent spikes may have belonged to the coxotrochanteral MRO. In the case of central recordings from the cell bodies of SR afferents, however, there is no such ambiguity. SR soma spikes were followed 1:1 by constant-latency EPSPs in levator motoneurones, and the delay of between 1.3 and 1.9 ms indicates that a monosynaptic connection is likely (Fig. 3). Although the number of central paired recordings from SR afferents and levator motoneurones is small, taken together with the results of spike-triggered averaging from nerve 3B5 recordings and cross-correlation analysis of afferent and efferent activity, the evidence suggests that levator motoneurones are directly excited by coxotrochanteral SR afferents. However, it is not yet certain whether the same applies to the single afferent neurone of the coxotrochanteral MRO.

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