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Tactile body raising: neuronal correlates of a 'simple' behavior in spiders

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Abstract
This review summarizes our recent results on the sense organs, the central nervous elements, and the neuronal mechanisms responsible for a relatively simple, tactile behavior of spiders. In *Cupiennius salei* (Keyserling 1877) (Ctenidae), a large tropical hunting spider, stimulation of tactile hairs on the ventral aspects of the body and the legs evokes reflex activity in several leg muscles. Coordinated contraction of these muscles raises the body - as in doing 'push-ups'. Using this reliable reaction we examined the neuronal circuitry underlying 'body raising behavior'. Electrophysiological recordings from particular leg muscles and from single, identifiable neurons in the leg ganglia reveal interneurons whose (electrical) activation causes the muscle reflexes. Depending on the exact stimulus situation (tactile and/or displacement stimuli), we have found local and plurisegmental responses and sequential activation of local and plurisegmental interneurons. The results provide a first glimpse of the architecture and functional hierarchy of single, sensory-motor elements in the fused central nervous system of spiders.

Key words: tactile hairs, motoneurons, local interneurons, plurisegmental interneurons, reflexes, leg muscles

INTRODUCTION

Comparative neurobiology seeks to understand the causal relationships between the nervous systems of various animal species and their behavior. Ultimately, it is expected that the comparative approach will yield results that can be generalized and applied across phyla. At the same time, the analysis of a specialized behavior pattern in a particular species can further our understanding of the functional possibilities and the limits of neuronal systems (Bullock 1984; Huber 1988). Neuroethological research with arthropods has been especially successful in understanding the roles played by single neurons and by relatively simple neuronal networks in controlling behavior (see, e.g., the work summarized by Burrows 1996). The success is due to the advantageous situation found in many arthropods (such as orthopteran insects and decapod crustaceans): large parts of their nervous systems are clearly partitioned into segmental ganglia; single, prominent neurons can be identified from individual to individual; and much of their behavior is relatively simple if not stereotyped (Hoyle 1977; Breidbach & Kutsch 1995; Katz & Harris-Warrick 1999).

Among the arthropods, spiders are interesting from a neurobiological point of view for at least two reasons: (i) Unlike the insect and crustacean species often studied by neuroethologists, spiders do not have a clearly seg-

mented and distributed nervous system; rather their central nervous system (CNS) consists of a fused ganglion complex that is concentrated in the prosoma (Fig. 5). (ii) Spiders use their hemolymph as a hydraulic fluid and extend their distal leg joints by local changes in hemolymph pressure. These two peculiarities of the basic arachnid body plan are interesting in themselves and in comparison with other arthropods. They do, however, also cause problems for experimental work with modern neuroanatomical and electrophysiological methods at the cellular level. The highly cephalized CNS makes orientation difficult for the neuranatomist, and the high hemolyph pressure existing in spiders (near the level of human blood pressure) precludes major dissection to isolate the CNS and hence restricts the possibilities for intracellular recordings from neurons and for dye-injections.

Faced with these peculiarities and difficulties we decided to follow a drastically reduced experimental approach. The present review will summarize experiments analyzing relatively simple leg reflexes involved in 'tactile body raising'. This is a stereotyped behavior of spiders that can easily be elicited in the laboratory. Our guiding principle and main question during this work has been: Can we identify the various neuronal components that control tactile body raising?

TACTILE HAIRS AND TACTILE BODY RAISING

Our experimental animal is *Cupiennius salei* (Keyserling 1877), a large Ctenid spider that we breed in the laboratory (Höger & Seyfarth 1995). *C. salei* is predominantly dark-active in its natural habitat in Central America; hence it has to rely mostly on mechanical and other non-visual clues to locate prey and to find its way about in the dark environment (Seyfarth 1980).

Like most spiders, *C. salei* has a dense coat of cuticular hairs covering the entire body surface. On the ventral aspects of the proximal leg parts, the hair coat is as dense as 400 hairs/mm^2

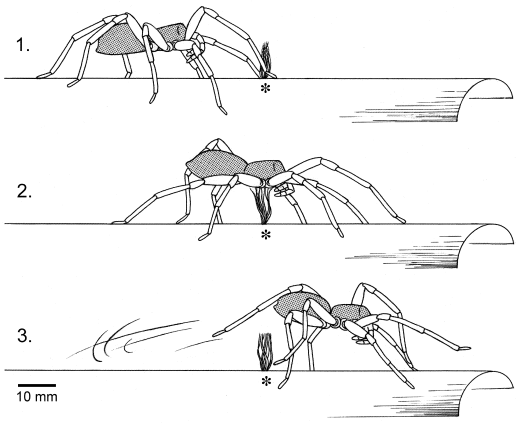

Fig. 1. Movement sequence (1. - 3.) of *C. salei* as it approaches and walks across 10-mm high wire obstacle on walkway. The spider raises its body as soon as it touches the obstacle (asterisk) with tactile hairs located ventrally on the sternum and on proximal leg parts (coxa, trochanter and proximal femur). [Drawings adapted from a series of photographs taken with electronic flash]

of body surface (Eckweiler & Seyfarth 1988). The shaft of these hairs is moveable and can be as long as 2.1 mm. The vast majority of cuticular hairs in spiders are 'tactile hairs' (Foelix 1985; Seyfarth 1985). Each is innervated by 3 bipolar neurons that are mechanosensitive and activated by direct touch deflecting the hair shaft in its socket. The exact sensory response (i.e., the electrical discharges of the 3 sensory neurons) depends on the direction and the intensity of the stimulus. The sensory discharges are conducted to the CNS via afferent axons, such as those running in the leg nerves.

When tactile hairs are touched, the spider will retract from the stimulus - either by quickly pulling away a leg or by turning around and walking off. However, touching the hairs located on the proximal ventral leg parts, the sternum, and the ventral opisthosoma will lead to a different, very distinct response. In this case, the animals abruptly raise their body by extending their legs. This happens through muscle contractions and through local increases in hemolymph pressure. We call this reaction 'tactile body raising' (Eckweiler &

Seyfarth 1988). In their natural habitat - *C. salei* is a swift hunter and lives on plants (Barth & Seyfarth 1979; Barth et al. 1988) - the behavior appears to protect the ventral body side against injuries (such as those inflicted by sharp thorns or kicking prey insects) that could cause fatal bleeding of the animals. In the laboratory, tactile body raising can be elicited very reliably and in a stereotyped fashion even at repeated stimulation. In addition to *C. salei*, we have observed stereotyped body raising upon stimulation of ventral hairs in large salticids (*Phidippus regius*), in theraphosids (*Brachypelma* sp.), and in 4 other *Cupiennius* species (Eckweiler & Seyfarth 1988). As further discussed below, deflection of just one hair of the many thousands present suffices to induce a coordinated extension of all 8 legs raising the body.

In order to study the behavior at three successive levels, we examine the tactile reaction in three different experimental situations: (i) in spiders freely walking across an obstacle; here we measure movement patterns and identify sense organs involved with the reaction; (ii) in animals that are tethered and walk on a spherical treadmill; here we record the electrical activity of muscles causing leg extension; and finally (iii) in completely restrained spiders to record neuronal events in the CNS with intracellular electrophysiological techniques.

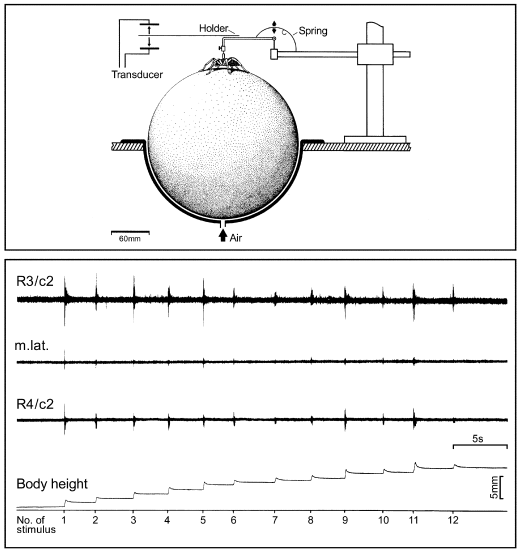

Fig. 2. Experiments on spherical treadmill. **Upper panel:** Arrangement for tethering spiders atop air-suspended styrofoam sphere. The spider (at its prosoma) is attached to a light holder that is pivoted and steadied by a weak spring; changes in body height are recorded via a capacitive transducer. **Lower panel:** Transient muscle activity during body raising. Upper 3 traces are simultaneous myogram recordings from c2-muscles in legs R3 and R4 and from lateral muscles (m. lat.). The latter contract to increase the hemolymph pressure in the prosoma. Bottom trace: transducer signal displaying increase in body height upon 12 successive tactile stimuli applied manually to several hairs at ventral trochanter of leg R3. Each stimulus evokes activity in all 3 muscles. [Adapted from Eckweiler and Seyfarth 1988]

THE LOCAL TACTILE RESPONSE IS FOLLOWED BY A PLURISEGMENTAL REATION

To determine the exact movement pattern during body raising, spiders were video-filmed while they walked along a narrow walkway and over a flexible, 10-mm high obstacle formed by a row of fine copper wires. The animals raise their body as soon as some of their ventral hairs brush against the wires; they then walk across the obstacle with extended legs as shown in Fig. 1. Ablation of hairs in various body regions confirms that body raising is induced primarily by the deflection of long hairs situated on the sternum and on the ventral surface of the proximal leg parts, i.e., of the coxa, trochanter, and proximal femur. Complete removal of all hairs in these regions leads to collision with the obstacle because the animals do not raise their prosoma until the hairy opisthosoma touches the wires (Eckweiler & Seyfarth 1988).

Our detailed analysis of the behavior from video-footage (filmed at 50 frames/s) shows that the spiders raise their body within 120-160 ms after first touching the obstacle. Consequently, the perception and central processing of tactile information as well as the coordination and execution of movements in the 8 legs must all be accomplished within this brief period.

For experiments under more controlled conditions spiders were tethered dorsally above an air-suspended styrofoam sphere as shown in Fig. 2 (top). In this situation, spiders can freely adjust their body height and walk (by rotating the sphere) but are 'fixed in space'. Changes in body height are recorded with a capacitive position transducer. Deflection of individual hairs (with a small wire loop) reveals that stimulation of a single, isolated hair suffices to elicit the concerted action of all 8 legs resulting in body raising (Eckweiler & Seyfarth 1988). Simultaneous deflection of several hairs (3 to 5 in a group) leads to successive, reliable reactions even when the stimulus is repeated at short intervals. An example of such repetitive action is shown in Fig. 2 (bottom).

In addition to exact tactile stimulation, the treadmill device also allows electrophysiological recordings of reflex activity in muscles bringing about body raising. We implant fine, flexible copper wires as electrodes in leg muscles and observe the appearance of muscle potentials reflecting the activity of several 'motor units' in each muscle ('electromyogram'; Figs. 2, 3). The signals are stored on magnetic tape and can be played back for later analysis. Such measurements show that the strongest and most reliable reflex response upon ventral tactile stimulation occurs in the coxal levator muscle c2 (muscle nomenclature according to Palmgren 1981). Contraction of this muscle pulls the coxa against the prosoma, presumably locking the pleural-coxal joint in place while the leg joints further distally extend via the hydraulic mechanism mentioned above. Fig. 2 (bottom) shows myograms recorded from adjacent legs (R3 and R4) during several successive body raising reactions on the sphere. Electrical activity sets in almost simultaneously in all muscles and is transient, that is, it occurs only during changes in body height. Apparently the spider can maintain its new position without continued electrical activity in the leg muscles. We assume that the 'residual tension' generally found after contractions in arthropod muscle and the newly adjusted hemolymph pressure

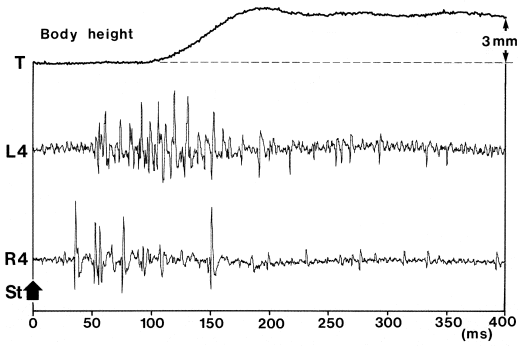

Fig. 3. Myogram recordings from c2-muscles in the two hindlegs (R4, L4), similar to Fig. 2 but displayed here at expanded time scale. Stimulation (St) of ventral tactile hairs at leg R4 elicits body raising (transducer signal, T). A burst of muscle potentials first appears in the stimulated leg (R4), and ca. 30 ms later in the contralateral leg. Each muscle recruits several motor units as indicated by the different amplitude and shape of potentials within the bursts. [Adapted from Kadel et al. 2002]

suffice to hold the body in the newly elevated position (Eckweiler & Seyfarth 1988).

Examination of such myograms at high time-resolution reveals how the muscle activities generated in several legs actually follow a finely tuned sequence. In the experiment shown in Fig. 3, 4 to 5 long hairs were touched beneath the coxa of the right hindleg (R4). Following a delay of ca. 30 ms after stimulus onset, muscle potentials first appear in the same leg being stimulated (R4), and then in the contralateral leg (L4) after an additional delay of 30 ms. Electrical activity in these and in the c2-muscles of all other legs causes muscle contraction and finally body raising - after a further 60-ms delay. The total latency of ca. 120 ms after stimulus onset corresponds to the value from our video-analysis of freely moving spiders crossing the wire obstacle.

The same sequence of events also applies when hairs on the forelegs are stimulated. Muscle c2 of the stimulated leg itself always reacts at least 25 ms prior to the musculature in the remaining, not-stimulated legs, but the latter are activated nearly simultaneously within a

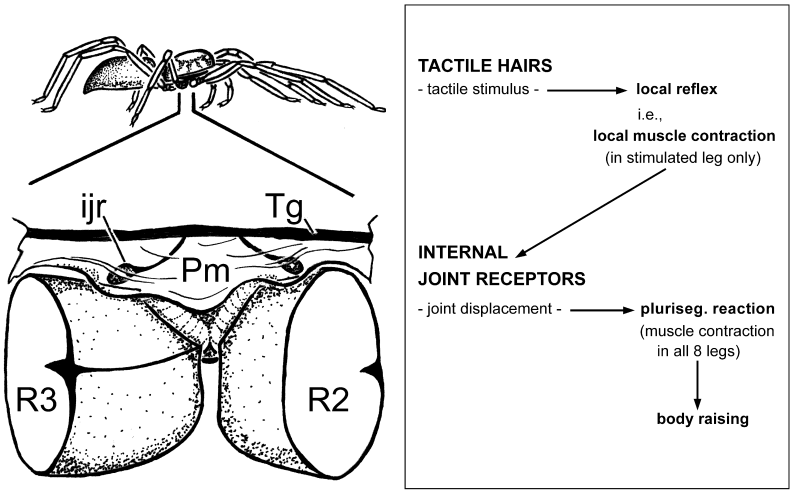

Fig. 4. Left: Topography of internal joint receptors in tergo-coxal joint of legs R2 and R3. Internal joint receptor (ijr) organs are located at the proximal edge of each leg coxa; they are comprised of several multipolar, mechanosensitive neurons which form small sensory ganglia underneath the pleural membrane (pm). These joint receptor organs are stimulated by coxal displacements. [Drawing adapted from Kadel et al. 2002] **Right:** Sequence of events leading from local reflex to body raising. Tactile hairs are involved at the first, local level, while internal joint receptors play a decisive role for initiating the next, plurisegmental stage (see text for detailed discussion).

period of 2 to 4 ms. Hence we identify a short-latency 'local reaction' in the stimulated leg itself, followed by a longer-latency 'plurisegmental reaction' that is virtually simultaneous in all remaining legs. The consistent time relation between stimulus onset and neuromuscular responses indicates that body raising behavior is largely determined by stereotyped reflex pathways.

What determines the sequence from local to plurisegmental reaction? Myogram recordings (such as the ones shown in Fig. 3) in combination with selective sensory ablations demonstrate that the plurisegmental response (that is, actual body raising) is only indirectly induced by the tactile stimulus. The decisive event for plurisegmental activation is displacement of the pleuro-coxal joint - which itself is brought about by short-latency local contraction of the c2-muscle (Kadel 1992; Kadel et al. 2002). If displacement of the joint is precluded experimentally (by immobilizing the joint with beeswax), tactile stimuli will merely result in a local reflex and not activate the remaining legs to raise the body. This is further supported by experiments with animals that are firmly restrained on their backs so that the experimentor can deflect individual hairs or selectively move single leg joints. There are two notable findings from such experiments: (i) Even under drastically reduced conditions the restrained spiders first react to tactile stimuli with a local reflex (confined to the stimulated leg), followed by the plurisegmental response in all remaining legs. (ii) Passive displacement of the pleuro-coxal joint alone (without prior tactile stimulus) directly induces plurisegmental activity. It turns out that internal joint receptors underneath the articular membrane of this joint perceive such movements (be they passive or active). Fig. 4 shows the topography of internal joint receptors in the tergo-coxal joints of two legs (R2 and R3). After surgical ablation of their sensory axons (through a tiny cut in the tergal membrane), the plurisegmental reaction fails to occur - both on the sphere and in spiders restrained on their backs. The plurisegmental response persists, however, after sham-operations that spare the sensory nerve (Kadel 1992; Kadel et al. 2002).

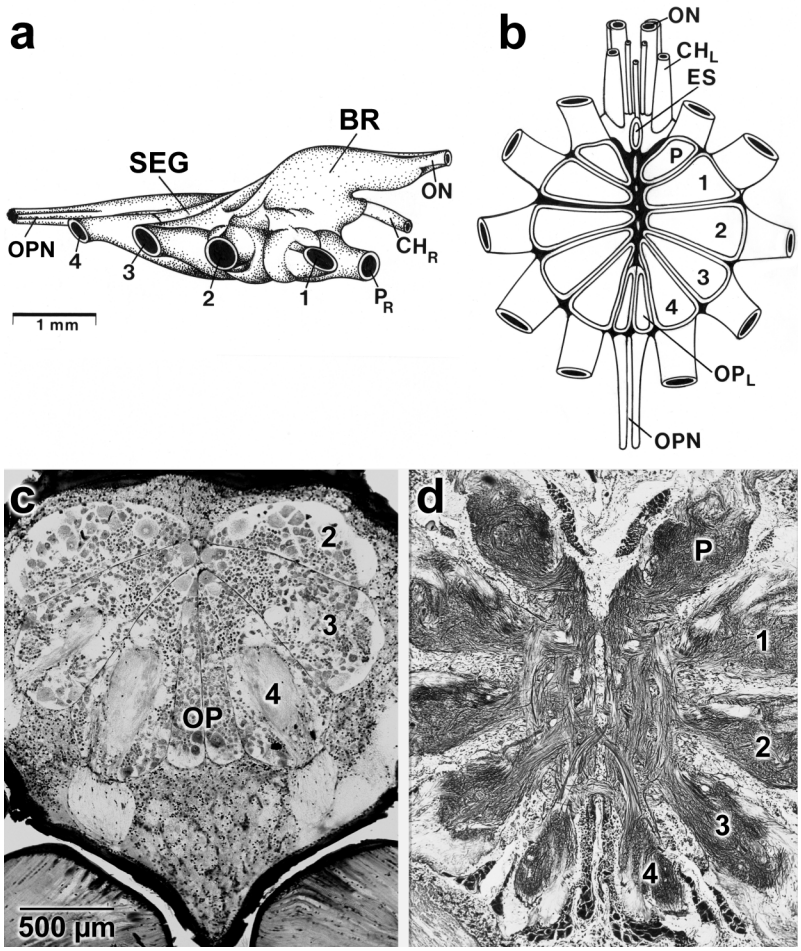

Fig. 5. Central nervous system of *C. salei*. **(a)** Schematized lateral view of subesophageal ganglion complex (SEG) and brain (BR). **(b)** Ventral view shows septal partitions dividing ventral SEG into serially arranged neuromeres. **(c)** Horizontal section through layer of neuronal cell bodies (cortex region) in ventral SEG; leg neuromeres 2 to 4 and smaller opisthosomal neuromeres (OP) are clearly separated by septa (thionine/Nissl stain; juvenile spider). **(d)** Horizontal section made further dorsally through SEG and stained with reduced silver technique; the septal partitions have disappeared; neuronal processes form a complex meshwork (neuropil) and fiber tracts that connect ipsilateral and contralateral regions. CH_R, CH_L: left and right cheliceral neuromere; ES: esophagus; ON: optical nerves; OP_L: left opisthosomal neuromere; OPN: opisthosomal nerves; P: pedipalpal neuromere; P_R: right pedipalpal nerve. [Drawings (a) and (b) modified from Babu & Barth 1984]

The diagram in Fig. 4 (right) summarizes these findings. We observe two successive reflex responses: (i) Touching hairs on the ventral, proximal leg parts evokes a local muscle contraction in the same leg. (ii) The resulting displacement of the coxa stimulates internal joint receptors in the tergo-coxal joint; this induces specific plurisegmental reactions in the other legs and finally body raising. Experimentally, we can directly evoke the plurisegmental response by passively moving the coxa. The two reflex responses appear to be identical in spiders standing on the spherical treadmill and in animals completely restrained on their backs. This latter finding is particularly important because it is the essential basis for our

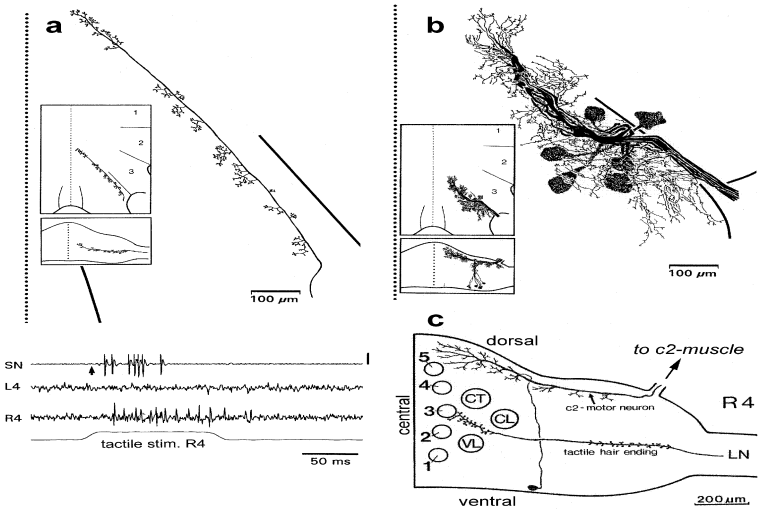

Fig. 6. Afferent and efferent projections in the SEG. **(a)** Primary tactile hair afferent. Top: arborization pattern in dorsal view, reconstructed from serially sectioned Lucifer Yellow preparation. Insets: small-scale dorsal and frontal views. Dotted line: longitudinal midline of SEG. Bottom: local response upon tactile stimulation of hairs in R4. Upper trace: the sensory neuron (SN) fires action potentials beginning 9 ms (arrow) after the hairs are touched (intracellular recording, high-pass filtered; scale bar: 5 mV); 2nd and 3rd trace: myogram recordings from c2-muscle in the two hindlegs; only R4 is activated. Bottom trace: signal driving tactile stimulator. [Adapted from Kadel 1992] **(b)** Motoneurons of c2-muscle in R4, dorsal view reconstructed from axonal backfills with nickel-chloride. The 6 somata are located in the ventral cortex layer (lower inset); the primary neurites ascend dorsally; their dendritic arborizations are confined to dorsal neuropil regions; leaving the CNS, the motor axons reach the c2-muscle via a small, separate nerve. [Preparation and reconstruction: Christiane Bickeböller] **(c)** Schematized frontal section through leg neuromere showing gap between tactile hair endings and c2-motoneuron; CT, CL, VL, and 1 - 5: position of longitudinal fiber tracts connecting ipsilateral neuromeres. [Modified from Milde & Seyfarth 1988]

analysis at the level of single central neurons discussed below.

ANATOMY OF THE SPIDER CNS AND INTRACELLULAR RECORDINGS

Before introducing individual neurons and their activities, I will briefly describe the gross anatomy of the central nervous system (CNS) in *C. salei*. As shown by the lateral view in Fig. 5a, the CNS is comprised of two main parts: (i) ventrally a relatively large subesophageal ganglion complex (SEG) that includes all nerve roots for the pedipalps, walking legs, and the opisthosoma, and (ii) dorsally a supraesophageal ganglion complex or 'brain' (BR) that provides nerves running to the 8 eyes and to the chelicerae. A ventral view of the SEG (Fig. 5b) demonstrates that the original metameric organization of the fused ganglion complex is still recognizable externally. The segmentally arranged hemiganglia (so-called 'neuromeres') are marked by ventral septa of connective tissue. Histological sections reveal the internal anatomy of the CNS. The majority of neuronal cell bodies are arranged in ventral cortex layers that remain largely separated by the segmental septa (Fig. 5c). Further dorsally in the SEG, the septal partitions between individual segments disappear, and we find a dense meshwork of neuronal processes and tracts of nerve fibers than run between ipsi- and contralateral neuromeres (Fig. 5d). Characteristically, the neurites profusely branch in mid-dorsal and dorsal neuropil regions, where they form synaptic contacts. Further details of the anatomy of the *Cupiennius*-CNS (and of the arachnid CNS in

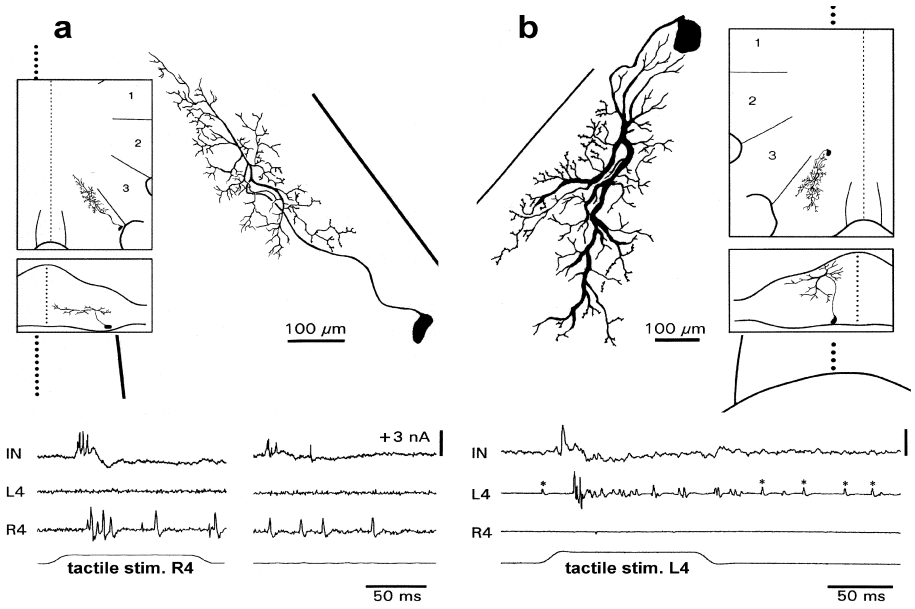

Fig. 7. Local interneurons and their activation following tactile stimuli; electrophysiological responses and anatomical reconstruction after intracellular recording and filling with Lucifer Yellow as in Fig.6a. **(a)** Spiking local interneuron in R4 characterized by arborizations that are confined to the antero-ventral part of the ipsilateral neuromere. Bottom left: tactile stimulus evokes local reaction in interneuron (IN) and in c2-muscle of R4. Bottom right: current injection (+3 nA) elicits action potentials in IN and activates a single motor unit in the ipsilateral c2-muscle. **(b)** Non-spiking local interneuron in L4; arborizations lie further dorsally than in spiking interneurons. Bottom: local reaction upon tactile stimulation; this interneuron (IN) generates only long-lasting, graduated potentials (scale bar: 10 mV); several neuromuscular units are activated in L4; one of them is tonically active due to the long-lasting depolarisation of the pre-motor IN. [Figures modified from Kadel & Seyfarth 2002]

general) are provided by Babu & Barth (1984), Babu (1985), and Seyfarth et al. (1993).

The electrical activity of individual neurons is recorded with glass microelectrodes that are inserted ventrally into the SEG through a tiny cut in the sternum. A small clot of hemolymph usually forms around the inserted electrode shaft securely sealing it in the opening. Using strong light sources, the septal partitions separating the neuromeres of the ventral SEG can be detected through the translucent cuticle (under a dissecting microscope) and are used as landmarks for positioning the electrode (Milde & Seyfarth 1988). For such intracellular recordings to be successful, the spiders must be completely restrained on a sturdy holder. The experimental setup includes custom-built devices for stimulation of tactile hairs and for controlled, passive displacement of individual leg joints. Following electrophysiological recordings of the responses to tactile and/or displacement stimuli, tracer substances (such as the fluorescent dye Lucifer Yellow) are injected into the neuron. Anatomical details of the labeled neurons are then reconstructed from serial histological sections (Milde & Seyfarth 1988; Kadel & Seyfarth 2002).

CENTRAL CORRELATES OF THE LOCAL AND THE PLURISEGMENTAL REFLEX RESPONSES

Intracellular recordings and anatomical reconstruction of labeled neurons reveal three different neuronal correlates of the reflex responses in SEG-neuromeres: (i) primary sensory terminals of the tactile hairs (= afferents), (ii) moto-

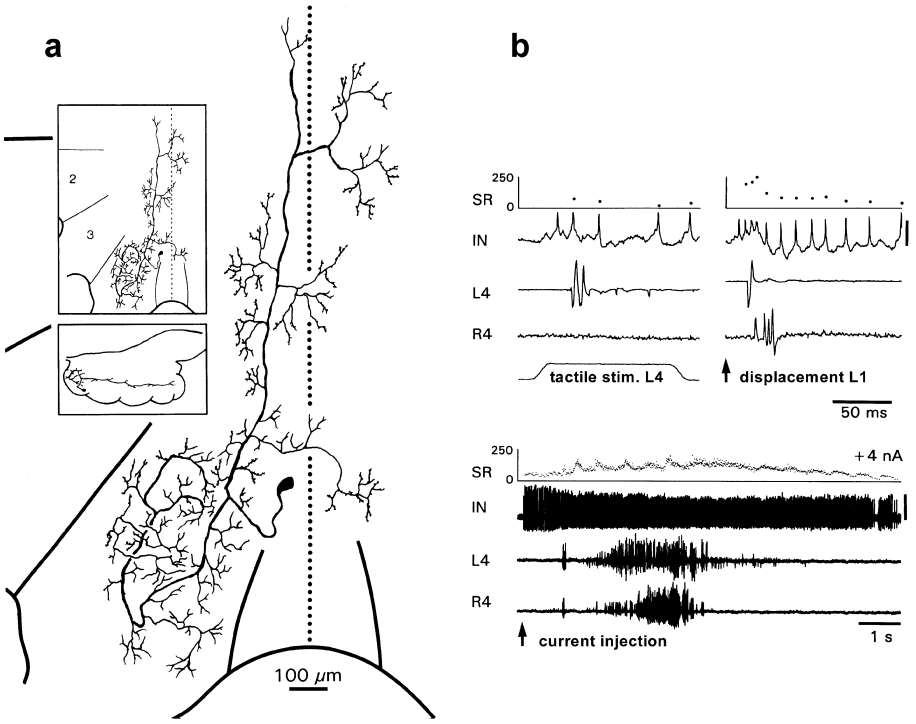

Fig. 8. Plurisegmental interneuron characterized by arborizations that extend into several ipsi- and contralateral neuromeres; electrophysiological responses and anatomical reconstruction after intracellular recording and filling with Lucifer Yellow as in Figs. 6a, 7. **(a)** The neuron arborizes extensively in L4 (which is also the recording site); a major neurite runs rostrad in parallel to the longitudinal midline (dotted), reaches into the 1st leg neuromere, and branches into contralateral neuromeres. **(b)** Left: local response to tactile stimulus in L4; maximum spike rate in IN is 60 spikes/s (SR, top trace). Right: plurisegmental response to coxal displacement stimulus (arrow) in L1; spike rate in IN rises up to 240 spikes/s. Bottom: responses to depolarizing current (+4 nA) injected into IN via recording electrode (vertical scale bar: 10 mV); the c2-muscles in both hindlegs (L4, R4) are activated simultaneously at an induced rate of ca. 100 spikes/s. [Modified from Kadel & Seyfarth 2002]

neurons activating muscle c2 (= efferents), and (iii) different types of interneurons that mediate between these afferents and efferents.

(i) Primary tactile afferents enter the SEG ventrally via the main leg nerve. They form numerous endings branching off the main neurite. Generally, tactile hair projections remain within the ipsilateral leg neuromer. Hence they are called 'local sensory projections'. Fig. 6a shows a typical example in leg neuromere R4. Upon tactile stimulation, the sensory unit (SN in Fig. 6a) is activated first. As expected, after a short delay the local motor response follows in the c2-muscle. There is no plurisegmental reaction because all joints have been immobilized in this experiment.

(ii) Unlike the situation found in vertebrates, muscle fibers of arthropods are typically innervated by several motoneurons ('polyneural innervation'; see Maier et al. 1987, for details of muscle innervation and the functional architecture of leg musculature in *C. salei*). In *C. salei*, muscle c2 receives innervation from at least 6 motoneurons. Their shape and location within the SEG are shown in Fig. 6b. Our experiments indicate that not all 6 units but only 3 to 4 are recruited during body raising (Bickeböller et al. 1991; Kadel et al. 2002).

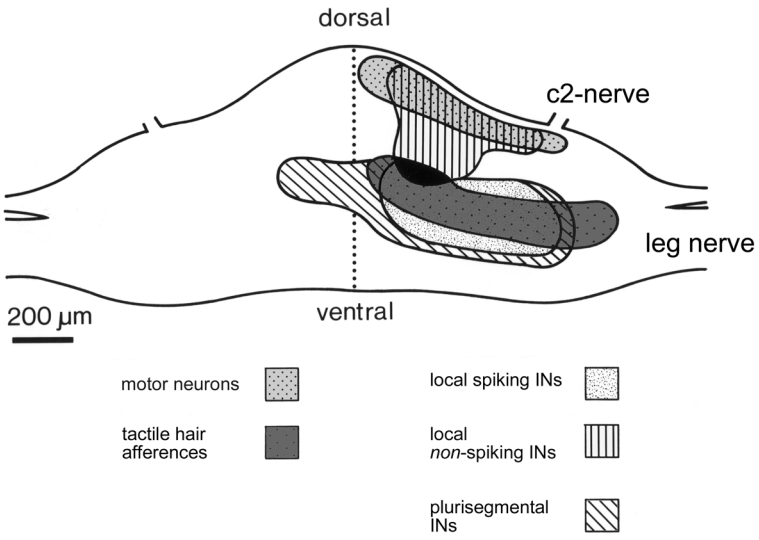

Fig. 9. Summary diagram of the 5 neuronal elements involved in body raising, their projections, and regions of overlap in a leg neuromere (schematized frontal view). Local non-spiking interneurons fill the gap between ventrally located hair afferents/spiking interneurons and the dorsal motor areas (see text for detailed discussion).

The dendritic projection areas of all c2-motoneurons resemble each other and are confined to the dorsal parts of the respective neuromere. Hence direct ('monosynaptic') contacts are not possible with the hair afferents that are located ca. 200 µm more ventrally. The anatomical gap becomes apparent in the frontal section through a neuromere in Fig. 6c. The relatively long latency of ca. 30 ms measured between stimulus onset and motor response is additional evidence for a lack of rapid, monosynaptic contacts in this reflex.

(iii) Various interneurons provide connections between the afferents and the c2-motoneurons. Depending on their electrical behavior and how far their projections reach into adjacent neuromeres, we distinguish local interneurons and plurisegmental interneurons (Milde & Seyfarth 1988; Kadel 1992; Kadel & Seyfarth 2002). Two different kinds of local interneurons are shown in Fig. 7a,b. The neuron in Fig. 7a responds with a burst of action potentials ('spikes') to a (standardized) tactile stimulus; as expected, the local motor reponse follows after a short delay. Interneurons of this type are called 'spiking local interneurons'. Generally, their arborizations are confined to ventro-medial parts of one leg neuromere (see lower inset in Fig. 7a). This example is especially interesting because a depolarizing current of +3 nA (injected into the neuron via the microelectrode) causes activation of a single motor unit in the c2-myogram. The finding suggests that this interneuron plays a significant role in the reflex pathway.

The neuron in Fig. 7b does not react with a burst of action potentials, but rather generates longer-lasting, graduated potentials in response to tactile stimuli. This is a 'non-spiking local interneuron'. Injection of depolarizing and hyperpolarizing currents into this neuron directly modulates the strength of the muscle response (data not illustrated in Fig. 7b). Characteristically, the arborizations of such non-spiking local interneurons extend from ventro-medial regions well into the dorsal-most parts of the leg neuromere. Consequently, direct contacts with motoneurons seem feasible here (see also Milde & Seyfarth 1988; Kadel & Seyfarth 2002).

The examples so far have only dealt with local interneurons and local reflexes. We also find various plurisegmental interneurons whose arborizations extend into several SEG-neuromeres; they are candidate neurons for mediating the plurisegmental reactions, that is, actual body raising behavior. Fig. 8 shows an example of a plurisegmental interneuron that is particularly important. Upon tactile stimulation in L4, the neuron responds with several

action potentials, and soon after, the local motor response follows in L4. Upon displacement of the pleuro-coxal joint in L1, however, there is a high-frequency spike discharge in the interneuron and a plurisegmental motor reaction (here shown only for L4 and R4). Depolarizing the interneuron with a current of +4 nA (instead of the joint displacement) also causes a massive spike discharge and a strong, plurisegmental motor response in L4 and R4 (Fig. 8b). In fact, by electrically manipulating the spike discharge of this interneuron we evoke motor reactions that greatly resemble the ones occurring during body raising. The arborizations of this type of plurisegmental interneuron are confined to the ventral part of the SEG, so that direct contacts with motoneurons are impossible but feasible via the non-spiking local interneurons discussed above.

Figure 9 summarizes our present data on the architecture of the different neuronal elements in a given leg neuromere. We assume that numerous tactile hair afferents converge onto relatively few interneurons, but that all interneuron types are present in multiple sets so that the whole system is redundant. The projection areas of the hair terminals, c2-motoneurons, and the three types of interneurons and regions of overlap are shown in schematized frontal view in Fig. 9. Based on the dorso-ventral extension of their neurites, non-spiking local interneurons alone are capable of providing local contact between tactile afferents and c2-motoneurons. Latency measurements of the various response times (data not illustrated here) in combination with our anatomical findings suggest the following sequence of neuronal events: In the case of a local reaction, the primary tactile hair afferents directly contact spiking local interneurons. Premotor, non-spiking local interneurons integrate this activity. As soon as a particular depolarization threshold is reached in these pre-motor elements, c2-motoneurons are excited in the same neuromere. In the case of plurisegmental reactions, we assume that afferent input from internal joint receptors (probably together with some tactile input) is rapidly distributed throughout the ventral SEG via plurisegmental spiking interneurons. Subsequently, and almost simultaneously in each segmental neuromere, the same local pathways are then utilized as described for the local reactions.

OPEN QUESTIONS AND PROSPECTS

The results reviewed here offer a first glimpse of the architecture and functional hierarchy of central neuronal elements responsible for a relatively simple reflexive behavior in spiders. At the same time new perspectives are opened for further research.

Our findings are in general agreement with the situation well known in insects and crustaceans. The sequence of information processing through the various types of interneurons corresponds with that found in the other arthropod groups - including the role of pre-motor, non-spiking local interneurons (see the discussions by Kadel 1992; Laurent 1993; Kadel & Seyfarth 2002). Of particular interest are plurisegmental interneurons of the kind shown in Fig. 8. This neuron responds to tactile stimulation at L4 and to coxal displacement at L1 by generating action potentials with different spike rates (maximum of 60 spikes/s upon tactile stimulation; more than 200 spikes/s at coxal displacement). Electrical manipulation of the neuron also causes a plurisegmental reaction that first appears at an induced activity of ca. 100 spikes/s. The electrical stimulus leads to simultaneous reflex activity in several motor units of both muscles, which closely resembles the situation seen in freely moving animals (while electrical stimulation of local interneurons generally activates merely a single motor unit). Electrical inhibition (by hyperpolarization) of this plurisegemental interneuron, however, does not preclude the motor response. Hence this interneuron behaves like a 'command element' in the CNS (Kupfermann & Weiss 1978; Edwards et al. 1999); it is active during the behavior, and its electrical excitation is sufficient but not essential for evoking the behavior pattern. We assume that the neuron is

part of a 'command system' consisting of several (redundant) plurisegmental interneurons, each of which can elicit the behavior alone. So far it is unclear how many elements comprise such a 'command system' in spiders.

Our experimental approach has focussed on tactile responses and hence has concentrated only on one detail in the behavioral repertoire of *C. salei*. Obviously, the animal uses the neuronal elements described here also in other situations and for other behaviors. So far we have not at all considered the role of sensory feedback ('reafference'), which is surely involved in controlling body raising but is totally precluded in fully restrained spiders (see also the discussions by Seyfarth & Bohnenberger 1980; Fabian-Fine et al. 1999, 2000). Moreover, so far we have relatively little information on internal joint receptor afferents, their projection pattern in the SEG, and their electrophysiological behavior. It is also important that we identify distinct functional compartments within individual local and plurisegmental interneurons. Detailed knowledge of the input and output zones along the main neuronal processes would allow more precise predictions about synaptic contacts between the various neuron types. Yet, further analysis of such neuronal circuitry in spiders requires a preparation in which the CNS can be dissected free for unobstructed access with microelectrodes. We have therefore begun to develop such preparations in which the hemolymph circulation is substituted by perfusion with saline.

So far our electrophysiological analysis has concentrated on the SEG-complex because this part of the CNS can be reached rather easily. We know very little about neuronal interactions between neurons in the SEG and so-called 'association centers' in the supraesophageal brain (via ascending and descending pathways). For instance, we observe that the tactile reaction habituates upon long-lasting, repeated stimulation, that is, the strength of the reaction decreases over time (Eckweiler & Seyfarth 1988). We assume that habituation and other 'context-dependent' behavioral adaptations are controlled by supraesophageal centers. Gronenberg (1990) has described interneurons descending from the brain that may play such a role. There is also recent evidence that neuromodulatory substances - such as the biogenic amines octopamine (OA) and serotonin (5HT) - affect the state of arousal and general excitability in *C. salei*. While it is still unclear where exactly these substances act in the nervous system of *C. salei*, we have identified and mapped numerous OA- and 5HT-immunoreactive neurons and their projections in the CNS (Seyfarth et al. 1990, 1993).

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