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Motor control of the mandible closer muscle in ants

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Abstract

Despite their simple design, ant mandible movements cover a wide range of forces, velocities and amplitudes. The mandible is controlled by the mandible closer muscle, which is composed of two functionally distinct subpopulations of muscle fiber types: fast fibers (short sarcomeres) and slow ones (long sarcomeres). The entire muscle is controlled by 10–12 motor neurons, 4–5 of which exclusively supply fast muscle fibers. Slow muscle fibers comprise a posterior and an antero-lateral group, each of which is controlled by 1–2 motor neurons. In addition, 3–4 motor neurons control all muscle fibers together. Simultaneous recordings of muscle activity and mandible movement reveal that fast movements require rapid contractions of fast muscle fibers. Slow and subtle movements result from the activation of slow muscle fibers. Forceful movements are generated by simultaneous co-activation of all muscle fiber types. Retrograde tracing shows that most dendritic arborizations of the different sets of motor neurons share the same neuropil in the subesophageal ganglion. In addition, fast motor neurons and neurons supplying the lateral group of slow closer muscle fibers each invade specific parts of the neuropil that is not shared by the other motor neuron groups. Some bilateral overlap between the dendrites of left and right motor neurons exists, particularly in fast motor neurons. The results explain how a single muscle is able to control the different movement parameters required for the proper function of ant mandibles.

Keywords: Muscle recording; Force measurement; Movement velocity; Muscle fiber types; Neuroanatomy; Insect

1. Introduction

In higher vertebrates as well as in arthropods, limb movements are of supreme importance in almost any behavioral context (e.g., escape, feeding, migration, etc.). Most natural behavioral sequences involve more than one limb, and limbs are generally controlled by many muscles. It is, therefore, a daunting task to analyze even simple natural behaviors in terms of their underlying motor programs and muscle contractions. Because of their relative simplicity, however, limb movements of arthropods provide interesting models for studying the motor control of behaviorally relevant movements (e.g., locust leg: Hoyle, 1974; Wolf and Burrows, 1995; Ott et al., 2000; stick insect leg: Bässler and Büschges, 1998; Akay et al., 2001). Jaw movements of ants are a case in point, as their mechanical organization is simple yet the jaws contribute to a complex behavioral repertoire.

Ants use their jaws as universal tools for prey catching, fighting, digging, leaf cutting, seed cracking or wood scraping, as well as delicate routines such as grooming, brood care, and food exchange or communication among nest mates (Hölldobler and Wilson, 1990). Accordingly, ant mandibles perform many different kinds of movements in terms of velocity, force output, and precision (Gronenberg et al., 1997). Based on a simple design, the mandibles of many ants are large and powerful (Janet, 1905; Snodgrass, 1935): a single-segmented appendage is attached to the head capsule by a hinge joint and is operated by a single pair of antagonistic muscles (opener and closer) on each side of the head. Mandibular force, velocity and dexterity mainly depend on the muscles that control the jaws. The mandible closer muscle is the key to the versatility of mandible functions. Its behavioral significance is reflected by its anatomical design; it is much larger than the opener muscle and occupies up to two-thirds of the entire head

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capsule volume. It is the largest muscle in any ant worker.

Ant mandible closer muscles generally comprise two distinct muscle fiber types that are composed of either short (2–3 μm) or long (5–6 μm) sarcomeres. Short sarcomeres suggest fast contraction properties while long sarcomeres imply slow yet forceful contraction of the respective muscle fibers (Costello and Govind, 1983; Silverman et al., 1987; Müller et al., 1992). In ant jaw closer muscles, fast muscle fibers always directly attach to the mandible closer apodeme (an analogue to the vertebrate tendon) whereas slow fibers attach either directly or via thin cuticular filaments to the apodeme. Within the mandible closer muscle, the three morphologically distinct fiber types (directly attached fast and slow fibers and the filament-attached slow type) are arranged in bundles that each consist of only a single fiber type (Gronenberg et al., 1997; Paul and Gronenberg, 1999; Paul, 2001). This is in contrast to the design of other arthropod muscles where functional properties may vary in a more graded fashion among fibers, and different fiber types may be arranged concentrically or interspersed within a muscle (Müller et al., 1992; Günzel et al., 1993). The segregation of different muscle fiber types within the ant mandible closer muscle hints at the possibility that those fiber bundles may represent functional units.

Little is known about the control of mandible closer muscles in insects, although they are prominent muscles in all biting and chewing insect taxa. Some aspects of mandible control have been studied in several species of trap-jaw ants (Gronenberg, 1995a; 1995b; Gronenberg and Ehmer, 1996; Gronenberg et al., 1993; 1997; 1998a; 1998b; Just and Gronenberg, 1998; Paul and Gronenberg, 1999), but the mandible mechanism of these ants is highly specialized. The innervation pattern of mandible muscles has been studied in locusts (Baines et al., 1990b), caterpillars of the hawk moth *Manduca sexta* (Griss, 1990), and the honeybee (Masuko, 1986; Rehder, 1989). Moth larvae only perform a reduced repertoire of mandible movements and honeybee mandibles are much reduced, reflecting phylogenetically derived sucking mouthparts. Hence bees, caterpillars or trap-jaw ants are not particularly well suited to study the general design of insect mandible control.

In the present study we investigate the control of ant mandible closer muscles. Our previous studies of mandible muscles (Gronenberg et al., 1997; Paul and Gronenberg, 1999) were based on morphological characteristics. However, structural properties such as sarcomere length do not always accurately predict physiological performance of muscle fibers in arthropods. Performance also depends on other factors such as innervation or enzymatic composition (Costello and Govind, 1983; Silverman et al., 1987). We therefore try to establish a causal relationship between the mandible movement and the electrical activity of particular muscle fiber types. A major question addressed is whether distinct muscle fiber bundles within a muscle can be activated separately, and how their action affects the resulting mandible movement. Furthermore, we try to correlate the number of physiologically distinct motor units with the number of morphologically identified motor neurons.

2. Materials and methods

Electrophysiology and mandible movement experiments were performed in Germany on *Camponotus rufipes* (Formicinae). Neuroanatomy was examined in Arizona on the local formicine species *C. festinatus* and *C. laevigatus*, and on the myrmicine species *Pogonomyrmex californicus* and *P. rugosus*. The arrangement of the muscle fibers of the three *Camponotus* species is virtually identical. The same is true for the two *Pogonomyrmex* species, which were included in the neuroanatomical study for comparative reasons (different ant subfamily). Like *Camponotus*, *Pogonomyrmex* rely on forceful mandible movements (seed cracking), generate similar mandibular velocities, and comprise the same classes of mandible muscle fibers in comparable positions as *Camponotus* ants (Gronenberg et al., 1997). Ants were kept in plaster-of-Paris nests under a 12h : 12h L : D cycle at 25 °C and 50% relative humidity. They were fed chopped cockroaches, crickets or wingless *Drosophila*, and honey-water (30%) or grains (*Pogonomyrmex*).

2.1. Electrophysiology

To assess the electrical activity of the mandible closer muscle, extracellular muscle recordings were performed on *Camponotus rufipes*. For anesthesia, ants were subjected to enflurane vapors (Ethrane, Abbot) for not more than five seconds and waxed onto a support so that the mandibles could move freely. Electrolytically sharpened stainless steel minutien pins (0.1 mm diameter) served as muscle electrodes. For differential recording, two pins per recording site (distance ca. 100 μm) were inserted through the cuticle into the mandible closer muscle using micromanipulators. In any given experiment, we simultaneously recorded from two of the four sites (Fig. 1a, 1b) and changed the combination of recording sites for each experiment. Recording sites were chosen so as to sample homogeneous muscle fiber groups (fast, slow and filament-attached slow fibers; Fig. 1a–c). While it would have been desirable to record from all fiber groups simultaneously, the animals were considerably impaired when more than two pairs of electrodes were inserted, and the resulting mandible movements were reduced and did not reflect natural activity. In contrast, animals with up to two carefully implanted electrode pairs were able

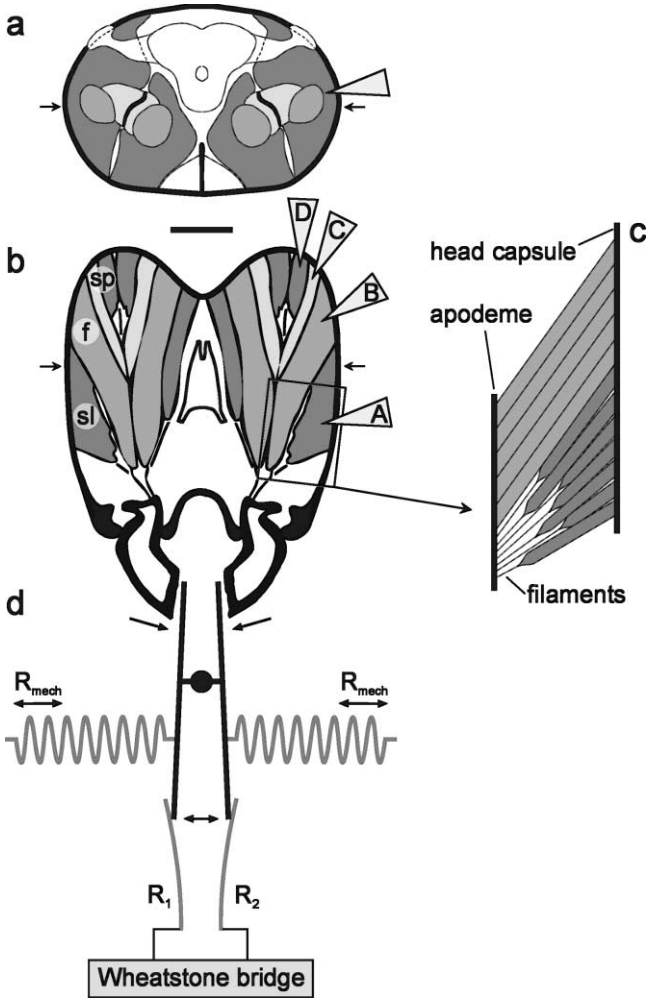

Fig. 1. Schematized composition of ant mandible closer muscle and experimental setup for electrophysiological experiments. Anterior view, a (plane indicated by arrows in b), and dorsal view, b (plane indicated by arrows in a; boxed area sketched in c), of a worker head of *Camponotus rufipes*; light grey directly attached slow fibers; medium grey directly attached fast fibers; dark grey filament-attached slow fibers; triangles A, B, C, D indicate electrophysiological recording sites corresponding to different muscle fiber groups [A: slow lateral (sl), B: fast (f), C/D: slow posterior (sp), C: slow posterior directly attached (spd), D: slow posterior filament-attached (spf)]; scale bar 500 μm. d: Mechano-electrical transducer used to record mandible movements. Lever arms transmit mandible movements to two strain gauges R1, R2 that change their electrical resistance upon bending. Strain gauges are part of a balanced Wheatstone bridge. Mandible movements result in currents across the bridge that are amplified and recorded. The mandibles contract against mechanical resistance R_{mech} which is set by adjusting two springs attached to the lever arms.

to move their mandibles as fast as ants unimpeded by electrodes (Gronenberg et al., 1997).

Muscle potentials were amplified up to 1.000 x (DAM 50, World Precision Instruments) and stored together with the record of the mandible movement (see below) on magnetic tape (Biologic DTR 1800). Recordings were off-line computer analyzed using appropriate software (Spike II, CED). After recording muscle activity, the recording sites were marked by two different methods: either by using the Prussian blue reaction (according to Wässle and Hausen, 1981; injection of positive current and precipitation of the ferric ions with potassium ferrocyanide solution) or by depositing a small amount of Congo red (Merck) at the electrode entry points followed by slightly moving the electrode backwards and forwards, thus transporting some of the dye into the muscle. After fixation (phosphate-buffered 4% formaldehyde or 2.5% glutaraldehyde, pH 6.9), the heads were dehydrated, embedded in Fluka Durcupan, and horizontally sectioned at 15 μm. Sections were then microscopically inspected.

2.2. Mandible movements

Force and velocity of mandible movements were recorded simultaneously with the muscle fiber activity in workers (head width: 3.5–4.4 mm) of *Camponotus rufipes*. A mechano-electrical transducer was constructed from two strain gauges (Measurements Group, Inc.) that served as resistors in a Wheatstone bridge and were connected to the mandibles by spring-loaded adjustable lever arms (Fig. 1d). The system allowed measuring mandibular movement against two preset mechanical resistances and was calibrated using standard weights. The resulting electrical signal was 0 (baseline) when the mandibles were open; upward deflection indicated mandible closing (cf. Fig. 3). The slope and amplitude of the transducer signal allowed calculating the mandible velocity and force, respectively. The slope was calculated from movement episodes that covered at least 20° (up to 40°) of mandible movement (see Fig. 3). Velocities measured with a low mechanical resistance were almost identical to those previously measured for unrestrained ants from video sequences (Fig. 8 in Gronenberg et al., 1997), indicating that the small mechanical resistance of the measuring device did not significantly slow down the mandible movements of these large ants. However, due to the nature of the device (mechanical resistance) we cannot rule out that the velocities reported here may differ from those an ant would perform with unloaded (empty) mandibles, even though our circumstantial evidence does not support this notion. While most mandible movements, and fast ones in particular, are symmetrical, ants occasionally perform slow movements with only one mandible (e.g., during grooming). The measuring device did not discriminate forces generated by the left or right mandible.

2.3. Neuroanatomy

To examine the mandible closer motor neurons' input regions within the subesophageal ganglion, the ants were anesthetized and immobilized in wax. A small window was cut into the head capsule of the ant laterally, pos-

teriorly, or dorsally to give access to a particular group of mandible closer muscle fibers. Some muscle fibers were then slightly damaged in order to disrupt the motor neuron terminals, and a tiny crystal of a fluorescent tracer (Fluoro Ruby, Molecular Probes) was placed onto the damaged parts using minutien pins or drawn-out glass capillaries. The hole in the head capsule was then sealed and the dye was allowed to be retrogradely transported by the damaged neurons into the central nervous system for 15–20 hours. The ants were then decapitated, their heads fixed in phosphate-buffered 4% formaldehyde, dehydrated with dimethoxy propane, embedded in Fluka Durcupan, and horizontally sectioned at 10–20 μm. Labeled motor neurons and stained muscle fibers were viewed under an epi-fluorescence microscope (Zeiss Axiophot) equipped with the appropriate filter combinations. Images were digitized (SPOT 2, Diagnostic Instruments) and graphically reconstructed using Adobe Photoshop software. Die-labeled motor neurons were compared with osmium-ethylgallate-stained material (fixation in 2.5% glutaraldehyde for 2 h, post-fixation in 1% osmiumtetroxide for 1–3 h, intensification in saturated aqueous ethyl gallate for 1–2 h).

3. Results

3.1. Muscle fiber groups and motor units

Ant mandible closer muscles comprise three types of morphologically distinct muscle fibers (see Introduction). In all ants studied, these different fiber types are segregated into bundles of like fibers that occupy characteristic positions within the head capsule. This general design is shown in Fig. 1a–c, which is based on the muscle fiber arrangement found in *Camponotus* (*Pogonomyrmex* shown in Gronenberg et al., 1997). While the relative and absolute size of each muscle fiber group differs across species and in some cases between individuals of different body size, the relative position of the different muscle fiber bundles is very similar in different species. The electrical activity of a particular muscle fiber type can therefore be assessed by inserting recording electrodes through the head capsule at specific locations (Fig. 1b). In each experiment, we simultaneously recorded from two of the three fiber types (e.g., recording sites A and B in Fig. 1b) or from the same fiber type in two different locations (sites A and D in Fig. 1b both probe filament-attached slow fibers). The dye marking procedures (Congo red and Prussian blue reaction) allowed the determination of the actual electrode position. In all cases the assumed electrode position coincided with the location of the dye precipitation.

During any particular recording experiment, potentials of different shape and amplitude were recorded within the same as well as across separate muscle fiber bundles. The amplitude varied considerably, particularly among potentials recorded from the fast muscle fiber type (Fig. 2c, 2d). These junctional potentials lasted 6–11 ms and have a spike-like appearance at low temporal resolution (as in Figs. 2, 3). We will refer to the potentials as

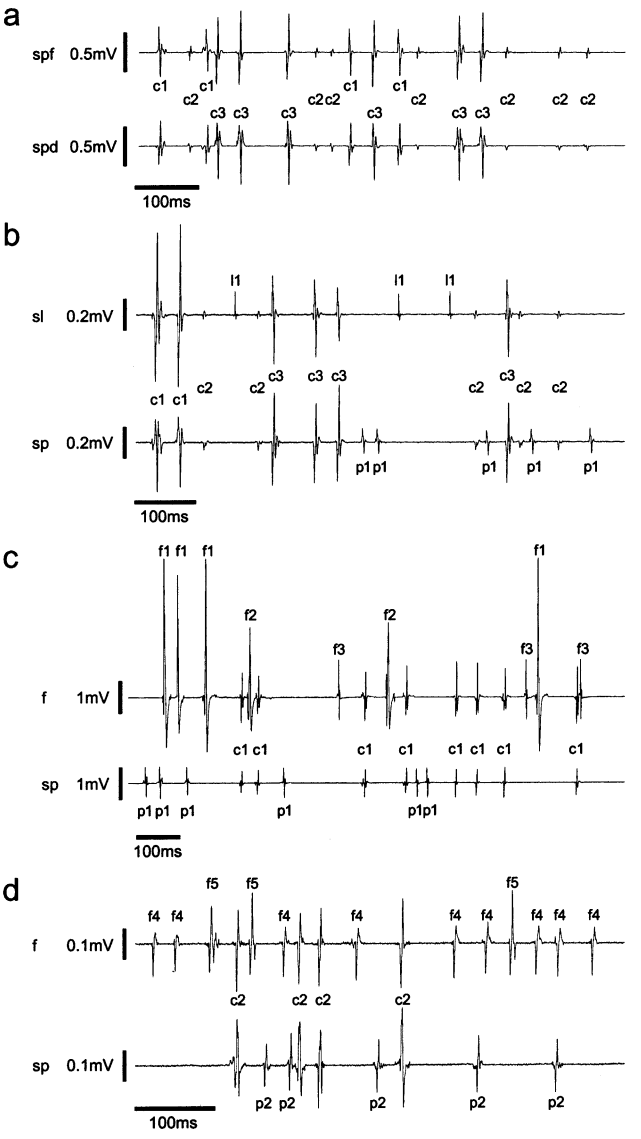

Fig. 2. Electromyograms of workers of *Camponotus rufipes* simultaneously recorded from two different muscle fiber groups of the mandible closer. a, b: examples from different animals showing slow posterior filament-attached (spf) and slow posterior directly attached (spd) fibers (a), and slow lateral (sl) and slow posterior (sp) fibers (b), respectively. c, d: two recording sequences from the same animal showing activity of fast (f) and slow posterior (sp) muscle fibers (mandible movement in c was faster than the one in d). Several unique spike types are labelled: ones exclusively recorded from fast muscle fibers f1–f5, from slow posterior fibers p1–p2, or from slow lateral fibers l1, respectively; and types simultaneously recorded from both traces c1–c3 ("c" therefore refers to spikes in both, the upper and lower trace). N = 3 (number of evaluated animals) for the combinations of electrode positions spf/spd, sl/sp, and f/sl, respectively, and N = 4 for f/sp; average recording time that was analyzed per animal = 25 min.

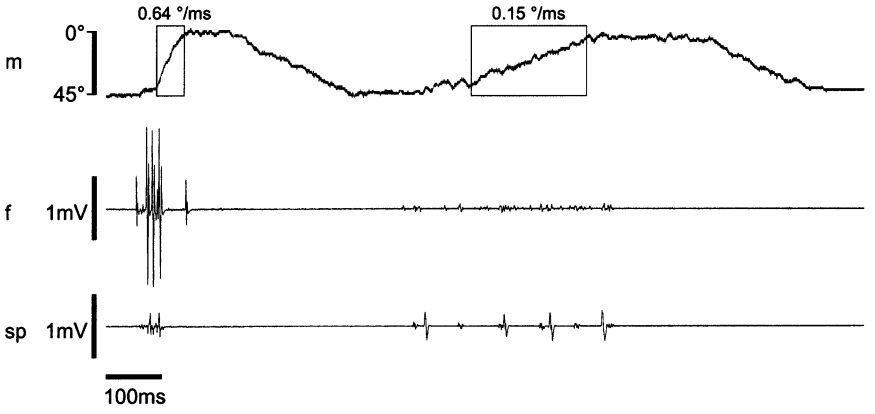

Fig. 3. Electromyogram and corresponding mandible movements of a worker of *Camponotus rufipes*. Top trace (m) represents mandible movements (baseline: mandibles open, inter-mandibular angle about 90°; maximum: mandibles closed; slope corresponds to movement velocity [°/ms]); rectangles indicate fast (0.64°/ms) and slow (0.15°/ms) movements and the respective integration times. Simultaneous muscle recordings from fast (middle trace, f) and slow posterior fibers (bottom trace, sp).

"spikes" even though they do not represent classical action potentials. Spikes in the same recording trace that were of identical or very similar shape and amplitude are referred to as a single spike type (each particular spike type is thought to reflect the activity in a single motor neuron). Thus, several spike types recorded with the same electrode indicate that the respective muscle fiber group is controlled by several motor neurons. An example is shown in the two traces of Fig. 2a, that were recorded from filament-attached (spf) and directly attached slow posterior muscle fibers (spd), corresponding to electrode positions D and C in Fig. 1b. Three spike types can be discriminated (Fig. 2a: c1–c3). The same potentials occur simultaneously in both recording traces. This temporal coincidence of spikes in two different muscle fiber groups is here interpreted as originating from activation of the same motor neuron that supplies both muscle fiber groups. Another potential explanation for the simultaneous occurrence of spikes in different recording channels is electrical crosstalk between the electrodes. However, we did not find any evidence for this artifact in our experiments. We found simultaneous spikes not only in adjacent muscle fiber groups, but also in ones much further apart from each other (e.g.. recording location "A" and "C" in Fig. 1; see Fig. 2b). There, potential crosstalk should be much reduced, but large simultaneous spikes can still be seen in both traces. Moreover, the large spikes (f1, f2) in Fig. 2c do not coincide with any potentials in the adjacent muscle fiber group (lower trace in Fig. 2c). If crosstalk were present, one would expect particularly strong artifacts (synchronous spikes in the lower trace) under these conditions, which we never found.

In general, no differences were found when recording activity from directly attached and from filament-attached slow muscle fibers in the posterior adjacent fiber groups. The functional similarity of these fiber types (directly attached and filament-attached slow fibers) is also suggested by functional morphology (Paul and Gronenberg, 1999). We will therefore disregard the mode of fiber attachment and only focus on the three functionally distinct fiber groups: fast fibers, lateral slow fibers and posterior slow fibers.

We found seven distinct spike types when simultaneously recording from the two separate packets of slow muscle fibers (the posterior and the lateral fiber group). One spike type was exclusively associated with the lateral muscle fibers (Fig. 2b: l1). Two spike types were exclusively recorded from the posterior muscle fiber bundle [only one type (p1) is shown in Fig. 2b]. In addition, four spike types occurred simultaneously in both fiber groups (three of them, c1–c3, are shown in Fig. 2b).

The top traces of Fig. 2c and 2d were recorded from a fast muscle fiber group in the same animal (corresponding to electrode position B in Fig. 1b). The sequence shown in 2c reflects a faster mandible movement than the one shown in 2d. Several spike types can be discriminated from the activity of this fast muscle fiber bundle. Of these, five unique spike types (Fig. 2c, 2d: f1–f5) did not coincide with any spikes in the bottom traces of Fig. 2c, 2d, which were recorded from slow posterior muscle fibers. Recording from fast muscle fibers generally revealed 4–5 unique spike types, indicating that this part of the muscle is controlled by at least five exclusive motor neurons that do not supply other parts of the muscle. In addition, spike types were found to occur simultaneously in fast and slow muscle fibers (e.g., the unmarked spike type in the upper trace of Fig. 2c that coincides with "c1" in the lower trace of Fig. 2c).

The posterior group of slow muscle fibers generally featured fewer spike types than the fast fiber bundle. In Fig. 2c, 2d (respective bottom traces), two exclusive spike types could be discriminated (p1, p2). In addition, three spike types (c1–c3) always occurred simultaneously in the upper and lower traces (c3 not shown

in Fig. 2c, 2d), indicating that they originated from the same set of motor neurons in both traces. The recordings in Fig. 2c, 2d, therefore, show that at least five motor neurons exclusively control the fast fibers, at least two motor neurons exclusively control the posterior slow muscle fiber group, and, in addition, three other neurons supply both muscle fiber groups simultaneously.

Different experiments did not always reveal the exact same number of spike types in a given muscle fiber group. The results of the ten experiments that showed the best resolution (highest number of spikes discriminated, high spike amplitudes and recording quality stable for at least one hour) are summarized in Table 1 (numbers reflect maximum numbers of spike types found in single preparations). These results indicate that the fast muscle fiber group is supplied by five distinct motor neurons, the posterior group of slow muscle fibers receives exclusive input from two neurons, and the lateral group of slow fibers is supplied by only one specific motor neuron. In addition, four motor neurons supply more than one muscle fiber group simultaneously. Judging by the number of recorded spike types, the entire mandible closer muscle therefore appears to be controlled by 12 motor neurons.

3.2. Force and velocity of mandible movements

When recording activity from the fast muscle fiber group, at least one particularly large spike type was found in every experiment. The amplitude of this particular spike type (2–6 mV; Figs. 2c, 3) was considerably higher than the amplitude of any spike recorded from the slow muscle fibers (less than 1 mV) or other spike types in the fast muscle fiber bundle. Whenever these large potentials occurred in the fast muscle fibers, the resulting mandible movement velocity was significantly higher compared to movements generated without the recruitment of this particular motor neuron (Fig. 3). The example in Fig. 3 also documents the finding that the large spikes generally (although not necessarily) occur together with smaller spikes. In Fig. 3, the mandible velocity (the slope of the upper trace that represents the mandible movement) is much higher during the first movement episode (0.64°/ms) than during the second one (0.15°/ms). The first movement is preceded by, and probably results from, the high-amplitude spikes in the fast muscle fibers. In contrast, the slower movement is only associated with small amplitude spikes in both, the fast and the slow muscle fiber group. Movement episodes preceded by high-amplitude spikes in the fast muscle fibers were always faster than movements lacking this particular spike type, even if the overall spiking frequency (number of total spikes per time unit) was higher than for movements accompanied by these large potentials.

The correlation between mandible movement velocity and the occurrence of the large spikes is depicted in Fig. 4. Those mandible movements that were preceded by the large-amplitude spikes were significantly faster ($p < 0.0001$; compare grey with black bars in Fig. 4, respectively) than mandible movements that were only accompanied by smaller spikes in the fast muscle fibers or that were not accompanied by fast muscle fiber activity at all. In the latter cases, the observed movements supposedly resulted from exclusive activation of slow muscle fibers. No significant correlation was found between the mandible movement velocity and the occurrence of particular spike types in any of the slow muscle fiber groups. This indicates that activation of fast muscle fibers by high-amplitude spikes is essential for generating maximum mandible closing movement velocities. For lower mandibular resistances (average load approximately 17 mN), the minimum mandible closing velocity at which the high-amplitude spikes started to occur was 0.37°/ms (mean velocity under these conditions = 0.49 ± 0.07°/ms, n = 14; cf. Fig. 4).

The fast mandible movements resulting from activation of the large-amplitude spikes are highly susceptible to increased load (Fig. 4). The mandible closing velocity correlated with the large spike activity in the

Table 1
Maximum numbers of unique spike types recorded in different muscle fiber groups of workers of *Camponotus rufipes*. Electrode positions correspond to Fig. 1b; n = 10 animals (3–4 for each combination of electrode positions). If the maximum number of spike types for a particular fiber group was different among single preparations with the same combination of electrode positions, numbers are marked as follows: ∗ in one preparation, only 4 different spike types were found for fast fibers (electrode positions A + B); ∗∗ in one preparation, only a single spike type was found for slow posterior fibers (electrode positions A + C/D and B + C/D, respectively).

Positions of electrodes	Spike types recorded in			
	Fast fibers exclusively	Slow post. fibers exclusively	Slow lat. fibers exclusively	More than one location simultaneously
B + C/D	5	1 - 2 ∗∗		3
A + B	4 - 5 ∗		1	4
A + C/D		1 - 2 ∗∗	1	4

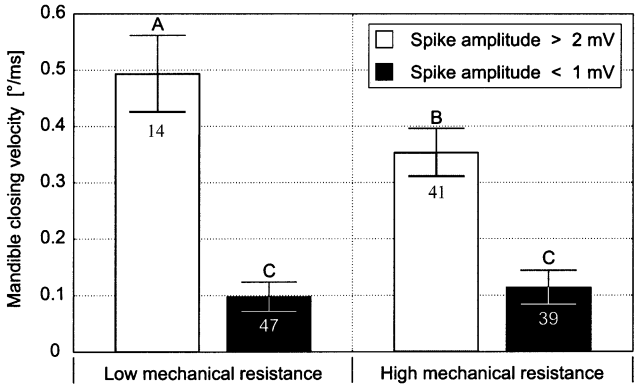

Fig. 4. Correlation between mandible closing velocity and electrical activity in muscle fibers with short sarcomeres ("fast fibers") in *Camponotus rufipes* at two different mechanical loads (low mechanical resistance: 10–27 mN; high mechanical resistance: 27–42 mN). Grey bars represent mandible movements preceded by high-amplitude spikes (>2 mV), black bars represent movements preceded by smaller (<1 mV) spikes or not associated with activity in the fast muscle fibers at all; bars depict means of n movement episodes evaluated ± standard deviation (n noted within the respective bar; N = 3 animals); statistically significant differences expressed by different letters [Kruskal–Wallis ANOVA test, H (3, n = 141) = 104.3136, $p < 0.0001$; Post hoc comparison, Scheffé test, Mann–Whitney U test, $p < 0.0001$; means of the three animals were also tested against each other (N = 3 for each bar), yielding the same statistical results ($p < 0.01$)].

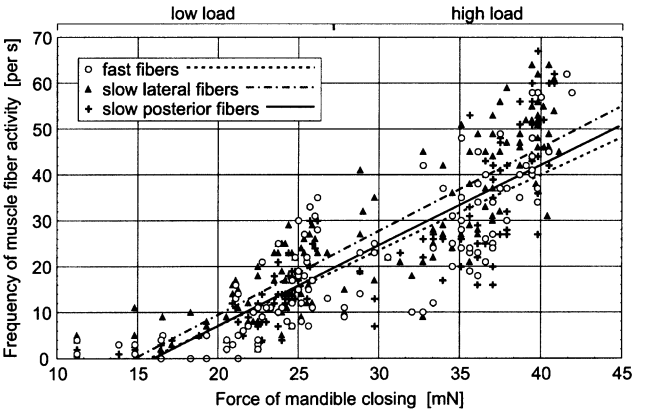

Fig. 5. Correlations between electrical activity and force developed by the mandible closer muscle. Each animal (*Camponotus rufipes* workers) was tested at two different mechanical resistances represented by the left (low load) and right half (high load) of the graphs. Each data point represents the spiking frequency recorded from a specific muscle fiber group during the corresponding movement episode. The three regression lines were calculated from separate data sets recorded from the three distinct muscle fiber groups (open circles: fast fibers, n = 115 movement episodes, N = 3 animals, Spearman r = 0.864, t = 18.201, $p < 0.01$; triangles: slow lateral fibers, n = 148, N = 3, r = 0.882, t = 22.628, $p < 0.01$; crosses: slow posterior fibers, n = 108, N = 3, r = 0.895, t = 20.600, $p < 0.01$). The data can statistically be described in terms of a single regression line: slopes are not significantly different [F = 1.0886 (df = 2, 365), p = 0.3378] nor are the intercepts [F = 0.6684 (df = 2, 367), p = 0.4868]; ANCOVA test.

fast muscle fibers is significantly reduced when the mandibles close against a higher mechanical resistance ($p < 0.0001$; compare grey bars in Fig. 4). In contrast, within the range of mandibular loads tested, no statistically significant differences were found for the slower mandible movements that result from activation of small-amplitude spikes in the fast and slow mandible closer muscle fibers (compare black bars in Fig. 4).

If the motor neuron(s) that generate the large amplitude spikes are specialized for fast contractions, how then is mandibular force output generated under conditions that require graded or maximum power output rather than ultimate velocity? In analogy to other motor systems, one would expect the spike frequency to control the contraction state of the muscle. An overall comparison of the spike frequency with the mandibular force (the amplitude of the mechano-electrical transducer output) shows a significant correlation between spike frequency and force generation (Fig. 5). This correlation holds for both fast and slow muscle fibers.

When comparing the activity of the three muscle fiber groups (fast, slow lateral, and slow posterior fibers; represented by separate regression lines in Fig. 5) with respect to the resulting mandibular force, we did not find significant differences. The data in Fig. 5 can statistically be described in terms of a single regression line (ANCOVA test; see figure legend for further details). This indicates that both fast and slow muscle fiber units increase their spike frequency simultaneously and proportionally when higher forces are generated. However, slow muscle fiber groups were sometimes activated exclusively without activating fast muscle fibers. For example, the four data points in Fig. 5 that show an activity of 0 Hz (open circles) indicate that fast fibers were completely inactive during these movement episodes. The large spikes in the fast muscle fibers occur only correlated with fast contractions (large spikes were not evaluated separately in Fig. 5).

As the mandible pivots around its axis in the mandible joint, the supposed lever arm and the relative direction of pull of the entire closer muscle at the apodeme basis might change during the mandible closing movement. It therefore seems possible that particular motor units may be activated depending on the mandible's actual angular position in order to maximize contraction efficiency. For example, lateral muscle fibers might reach their optimum angle of pull when the mandibles are almost closed whereas posterior muscle fibers might contribute much less force under these conditions. To test this hypothesis, we compared the spiking activities in posterior and lateral muscle fibers for different mandibular angles over a range of 45° (covering most of the mandibles' working range in *Camponotus*). When plotting the spike frequency of slow lateral and posterior fibers against the mandibular angle (analogous to Fig. 5), no significant differences between these fibers were found. Instead, both data sets can statistically be described in terms of a single regression line {ANCOVA test: slopes are not

significantly different [F = 0.0597 (df = 1, 252), p = 0.40] nor are the intercepts [F = 3.6509 (df = 1, 253), p = 0.07]}. The spiking frequency thus indicates that posterior and lateral muscle fibers are not recruited differentially. Hence, we did not find any indication for predictable recruitment sequences of the different muscle subunits. However, this does not exclude the possibility that specific spike classes may be co-activated at particular mandibular angles or force requirements. Moreover, distinct muscle fiber groups were activated independently of each other in some individual cases.

3.3. Neuroanatomy

We found several mandible closer motor neurons to be activated independently of each other. Hence, they probably receive different synaptic input and may therefore differ morphologically in their dendritic input regions. We have analyzed the anatomy of mandible closer motor neurons from eight successful tracing preparations of *Camponotus* (*C. festinatus*, *C. laevigatus*) and nine preparations of *Pogonomyrmex* (*P. californicus*, *P. rugosus*). No substantial differences (number of neurons, position of cell bodies, primary arborization pattern of dendrites) have been found among these species. We therefore assume that the neuroanatomical findings also apply to *C. rufipes*, which we used for the physiological and movement measurements.

Mandibular motor neurons originate in the subesophageal ganglion and their axons project through the mandibular nerves (Fig. 6a, 6b) toward the mandible closer muscle. The mandibular nerves are the most prominent nerves of the subesophageal ganglion. They are mixed nerves and, in addition to the mandible closer motor axons, carry the opener muscle motor axons and the sensory axons that originate from sensilla on the mandible (indicated in Fig. 6a, 6b). Our ehtylgallate preparations show that the mandible closer motor axons are the thickest neurons entering the subesophageal ganglion (5–8 μm) and they can be identified by their axon diameter in ethylgallate-stained material (Fig. 6a, 6b). The mandible motor neurons are restricted to the mandibular neuromere, the ventral-most part of the ganglion (the subesophageal ganglion is composed of the labial, the maxillary and the mandibular neuromeres). The cell bodies of the mandible closer motor neurons are large (diameter 20–25 μm) and reside in anterior and antero-lateral parts of the cell body rind of the ganglion (Fig. 6c–f). The dendrites are restricted to the anterior half of the ganglion (compare Fig. 6a with c–f) where they overlap with sensory afferents from the mandibles (Fig. 6a, 6b). All mandibular sensory afferents together supply a larger part of the subesophageal ganglion than do the mandibular motor neurons [revealed by anterograde staining of mandibular afferents (Gronenberg, unpublished; evidence for other ant genera: Gronenberg et al., 1998a; 1998b)]. Unlike the motor neurons, some of the mandible afferents pass through the cervical connectives into the thoracic ganglia (not shown in the figures).

Mandible closer motor neurons are mainly restricted to the ipsilateral side of the ganglion. In most neurons, only some fine dendritic branches cross the midline and reach into contralateral neuropil for a short distance (Fig. 6d–f). This central region of bilateral overlap is largest in those motor neurons that supply the fast closer muscle fibers (Fig. 6c). In addition, preparations in which the fast muscle fibers were labeled usually showed the highest number of cell bodies (five and six, respectively, in Fig. 6c). However, in five preparations, up to three of the labeled motor neurons were not specific for this particular muscle fiber group but would also be stained when labeling a different set of muscle fibers, indicating that these motor neurons supply more than one muscle fiber type.

When the lateral set of slow muscle fibers was stained, generally only two motor neurons were labeled (Fig. 6d). These particular motor neurons are characterized by lateral dendritic branches in addition to the central branches common to all mandible closer neurons. The cell bodies of these neurons with processes in the lateral neuropil are situated in a lateral soma cluster close to the root of the mandible nerve (Fig. 7).

In Fig, 6e, slow lateral muscle fibers were stained together with a few of the adjacent fast fibers. Only two of the "fast" motor neurons were stained together with three neurons that control the lateral slow muscle fiber group. The two "fast" motor neurons give rise to the dendrites that characteristically cross the midline. Two of the "slow" motor neurons are characterized by their postero-lateral dendritic arborizations and by their lateral soma location as seen in Fig. 6d and described in the previous paragraph (the third lateral cell body probably belongs to a neuron that supplies all slow muscle fibers, as it was also found after tracer application to the posterior muscle fiber group shown in Fig. 6f).

Staining from posterior muscle fibers revealed three motor neurons that lack the postero-lateral dendrites and whose central dendritic branches barely cross the midline (Fig. 6f). Two of their cell bodies are found in the anterior clusters and a third one resides more laterally close to the mandibular nerve root. The latter is probably identical with one of the cell bodies in Fig. 6e and represents a motor neuron that supplies all the slow mandible closer muscle fibers simultaneously.

The anatomical data are summarized in Fig. 7. Together, at least ten different somata were identified to supply the mandible closer muscle. Four of these (numbers 5, 6, 8, 9) were reliably associated with the fast muscle fibers. One or two somata give rise to motor neurons supplying lateral slow muscle fibers (number 2 and one of its neighbors, either number 1 or 3), and 1–2 cell bodies exclusively supply posterior slow muscle

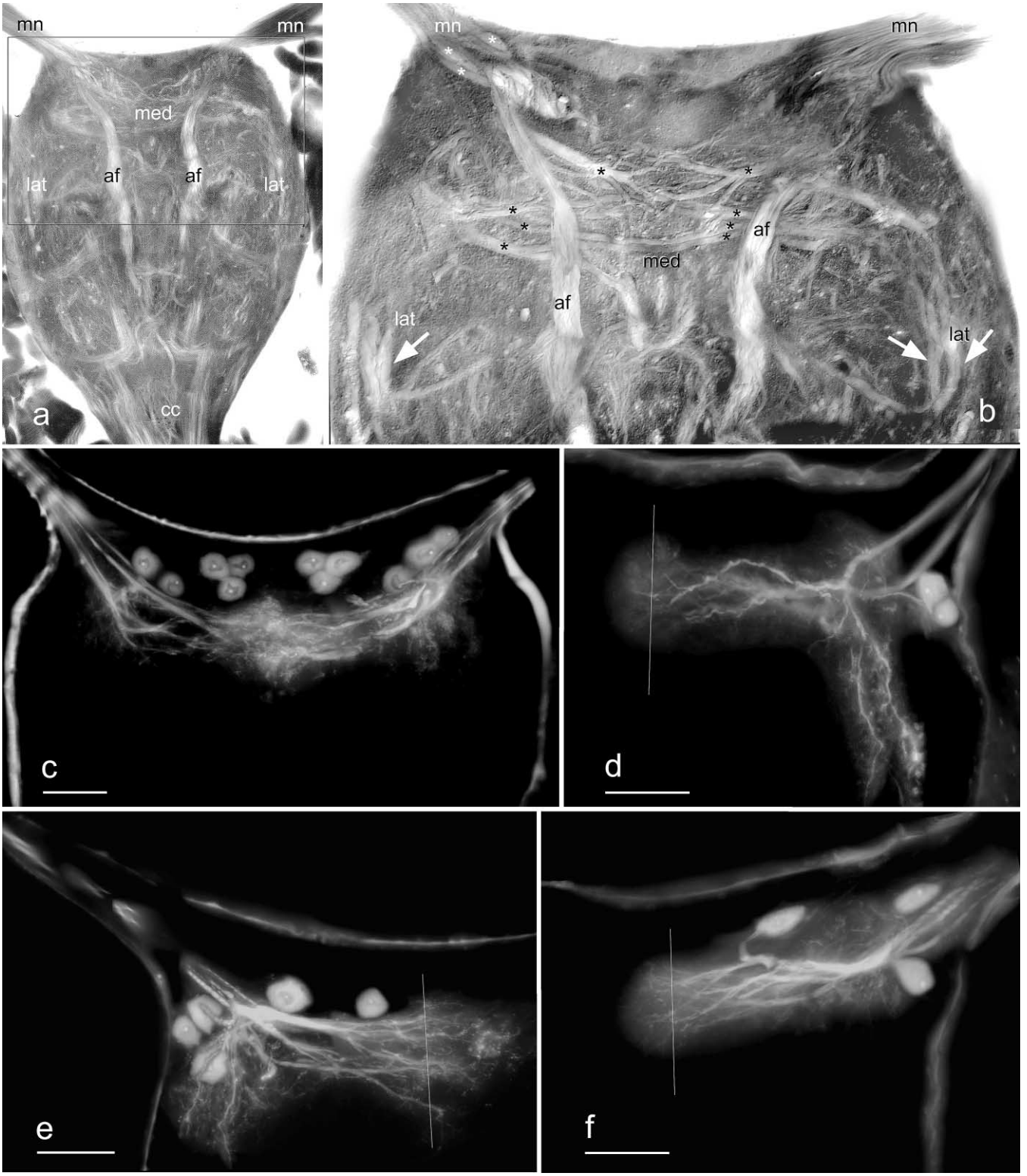

Fig. 6. Ethylgallate-stained (a, b) and Fluoro-Ruby-stained (c–f) mandible closer motor neurons in the subesophageal ganglion of *Camponotus laevigatus* (a–c, e) and *Pogonomyrmex rugosus* (d, f); boxed area in a enlarged in b; motor neurons stained by tracer application in fast muscle fibers (left side of c), in fast and slow posterior fibers (right side of c), in slow lateral fibers (d), in fast and slow lateral fibers (e) and in slow posterior muscle fibers (f); af mandibular sensory afferents; cc cervical connectives; lat lateral and med medial mandibular motor neuropil; mn mandibular nerve; arrows point at lateral motor neuron dendrites, black asterisks indicate motor neuron primary dendrites, white asterisks indicate motor axons in nerve; midlines indicated by white lines in c–f; scale bars 50 μm; anterior is up.

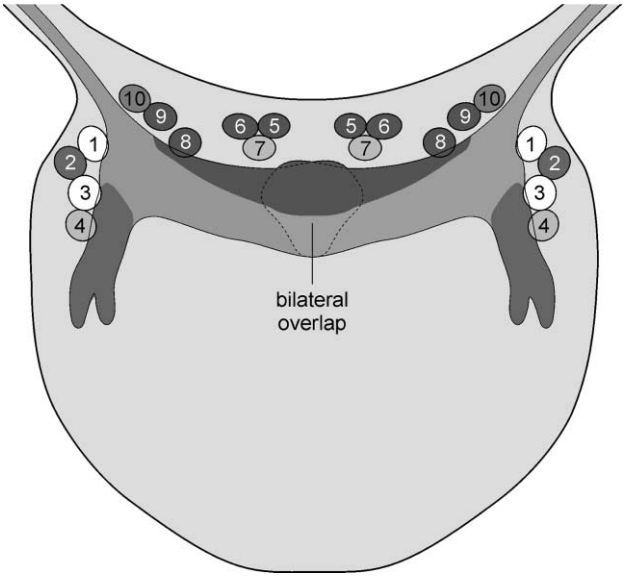

Fig. 7. Schematic showing subesophageal ganglion with dendritic regions and cell body locations of mandible closer muscle motor neurons (horizontal plane, anterior is up). Cell bodies labeled 5, 6, 8, 9 supply fast muscle fibers exclusively; 2, 10 supply slow muscle fibers (2 supplies lateral, 10 posterior ones); 1, 3 supply either lateral or posterior slow muscle fibers, or both groups; 3–4 motor neurons innervate all muscle fiber types simultaneously (among them 4 and 7). Different sets of motor neurons share most of their input region (light grey); distinct input regions of fast (dark grey) and slow lateral muscle fibers (medium grey); bilateral overlap is largest in fast motor neurons.

fibers (number 10 and one soma in the lateral cell body cluster). In addition, we found 3–4 cell bodies (among them numbers 4 and 7) that were revealed in many preparations irrespective of the muscle fiber group stained. These motor neurons supposedly supply all muscle fiber types simultaneously. Cell bodies number 1 and 3 were hard to differentiate because they were stained in different combinations with other neurons. They supply either lateral or posterior slow muscle fibers, or both groups.

The dendrites of motor neurons supplying fast muscle fibers are situated slightly more anterior compared to those of the other motor neurons, and they project deeper into contralateral neuropil. Neurons supplying lateral slow muscle fibers, in addition to their central dendritic collaterals, feature postero-lateral dendrites. However, the dendrites of motor neurons that supply posterior slow muscle fibers could not be discriminated from the central dendrites of motor neurons that supply the entire muscle (Fig. 7). In conclusion, all mandible closer motor neurons strongly overlap in the antero-median mandibular neuropil. We found additional, distinct dendritic branches only in motor neurons that supply either fast or lateral slow muscle fibers.

4. Discussion

4.1. Motor units and their input regions

Arthropod motor neurons generally form multiple synaptic sites on each of their postsynaptic muscle fibers (Hoyle, 1974). As most arthropod muscle fibers do not generate action potentials, this design ensures a rapid activation of the muscle fiber by the motor neurons. Therefore, the potentials recorded from a muscle reflect the activity of the motor neurons that supply it. This allows the identification and characterization of the motor neurons supplying a given muscle by its electromyogram (Hill and Govind, 1983; Rathmayer and Erxleben, 1983; Clarac et al., 1987; Bauer and Gewecke, 1991; Kawasaki and Kita, 1995; Rathmayer, 1996). The number of muscle spikes recorded from the mandible closer muscle in different recordings was slightly variable in the present study. Because of the restricted number of simultaneous recordings (see Materials and methods) some ambiguity remains when assigning types of potentials (motor neurons) to specific muscle fiber groups. The recording experiments imply that the closer muscle is supplied by 9–12 motor neurons (Table 1). Other possible explanations requiring fewer motor neurons would exclude the existence of a motor neuron that exclusively supplies the lateral slow muscle fibers. This would contradict our neuroanatomical data (Figs. 6d, 7). Neuroanatomically, at least 10 motor neurons could be discriminated by their cell body location and dendritic arborization pattern (Fig. 7). By superimposing morphological and electrophysiological data, the following innervation pattern emerges for the mandible closer muscle: 4–5 motor neurons exclusively supply the fast muscle fibers, 1–2 neurons control the posterior group of slow muscle fibers, and 1–2 neurons supply the anterolateral slow fibers. In addition to these specific neurons, 3–4 motor neurons innervate the entire closer muscle. These motor neurons therefore control all types and locations of muscle fibers.

Ant mandible motor neuron dendritic fields do not completely overlap in the subesophageal ganglion. The partial separation of their dendritic fields indicates that they receive common as well as individually distinct input in specific regions of the neuropil. While more detailed physiological and ultrastructural analyses would be required to resolve this question, differences in the motor neurons' dendritic input regions suggest that the different fiber groups may be activated independently by some of the motor neurons. This is supported by the occurrence of unique muscle potentials specific for each of the respective muscle fiber bundles (Fig. 2) and by particular mandible movements during which specific muscle fiber groups were completely inactive. We conclude that functionally distinct (fast and slow) and spati-

ally separated (slow posterior and lateral) muscle fibers can be recruited individually.

The larger region of bilateral dendritic overlap found in motor neurons supplying the fast muscle fibers indicates that bilateral input may be more important for the control of fast movements. The slow and precise "manipulation" of objects may not require a precisely synchronized bilateral action and may depend more on feedback from ipsilateral mandibular sensory input (hair receptors, campaniform sensilla and mandibular muscle receptor organs; Gronenberg et al., 1998a). The idea that grasping movements that are too fast for sensory feedback depend more on bilateral input is supported by findings in trap-jaw ants. In *Odontomachus*, the fast mandible motor neurons receive bilateral sensory input and always act in synchrony (Gronenberg et al., 1993; Gronenberg, 1995b; Just and Gronenberg, 1998). The somata of the trap-jaw ants' fast motor neurons occupy positions similar to that of cell bodies 5 and 6 in *Camponotus* and *Pogonomyrmex* (Fig. 7). This similarity of fast mandible closer motor neurons supports the idea that the trap-jaw mechanisms are homologous to, and can easily be derived from, the general design of mandible muscles and motor neurons in less specialized ants such as *Camponotus* or *Pogonomyrmex* (Gronenberg and Ehmer, 1996).

4.2. Multineuronal innervation and muscle modulation

In insects, the number of motor neurons supplying a single muscle varies from only one (in a small neck muscle: Strausfeld et al., 1987) to 3–6 in different leg muscles (Hoyle, 1974; Walther, 1980; Burrows, 1996) and 5 in antennal muscles (Bauer and Gewecke, 1991), to 12 in a caterpillar mandible muscle (Griss, 1990). Mandibular muscles appear to be controlled by a particularly high number of motor neurons not only in ants (10–12 in the current study) and caterpillars but also in honeybees (6–8; Rehder, 1989). Many motor neurons may be required to generate different forces and velocities in this uni-segmental limb. Other insect mouthparts or legs comprise several segments. Their movement is thus controlled by many muscles which together can be orchestrated by a larger pool of motor neurons (about 70 motor neurons in the locust hind leg; Burrows, 1996). The contraction properties of arthropod muscle fibers depend on the type of neuron by which they are activated (Hoyle and Burrows, 1973). Different motor neurons supplying the same muscle fibers generally serve to modulate the contraction properties of those fibers. The two mandible closer muscle fiber types in ants ("fast" and "slow") can thus probably be finely tuned by activity in the different motor neurons supplying them. We assume that some of the mandible closer motor neurons in ants release modulatory transmitters. Locust mandibular muscles receive innervation from serotonergic neurons (Tyrer et al., 1984; Baines et al., 1990b), occupying similar positions within the subesophageal ganglion as the somata of some motor neurons stained in the present study. Some other locust mandible closer motor neurons release proctolin (Baines et al., 1990a) which may enhance muscle contraction without an increase in spike frequency (Allgäuer and Honegger, 1993; Bartos et al., 1994). In addition, some insect muscles are modulated by dorsal unpaired median (DUM) neurons that contain octopamine (Hoyle et al., 1974; Orchard et al., 1989). Such neuromodulatory substances may be involved in the fine tuning of mandibular movements in ants as well, but no immunocytochemical data are available for ant nervous systems.

Some crustaceans and insect skeletal muscles are also modulated by common inhibitor motor neurons (Pearson and Bergman, 1969) that supply several muscles simultaneously and release GABA as their neurotransmitter (e.g., locust flight steering muscle: Wolf, 1990; antennal innervation of crickets: Honegger et al., 1990; Allgäuer and Honegger, 1993). However, preliminary experiments did not reveal any evidence for GABA-like immunoreactivity of mandibular muscles and motor neurons in the ant *Odontomachus* (Stefan Just, personal communication). Likewise, mandibular motor neurons in adult and larval moths, *Manduca sexta*, do not contain GABA (Homberg et al., 1987; Griss 1990). We therefore assume that mandible muscles in ants, and probably in insects in general, are not controlled by common inhibitory motor neurons.

Besides modulation, fast muscle contractions may require simultaneous activity in several fast motor neurons to release a sufficient amount of transmitter to rapidly depolarize the entire set of fast muscle fibers. Fast mandible muscle fibers of trap-jaw ants (genera *Odontomachus* and *Anochetus*) are supplied by two particularly thick motor neurons. Each of these two fast neurons makes synapses with only half of the fast muscle fibers even though the entire set comprises less than 50 fibers which always act in a highly synchronized way (Just and Gronenberg, 1998). If this design was common in ants in general, the 4–5 fast motor neurons found in the present study might not all converge on the same muscle fibers. Rather, each individual fast muscle fiber might be supplied by only a subset of those fast motor neurons.

4.3. Force and velocity of mandible movements

We found that the fastest mandible movements always coincide with large spikes in the fast muscle fibers. In the absence of these large spikes, the resulting mandible velocity was reduced by a factor of five (Fig. 4), even if small potentials were present in the fast muscle fibers. Fast mandible movements therefore result from rapid contraction of muscle fibers with short sarcomeres, which corroborates our use of the term "fast" muscle

fibers. The maximum angular velocities determined in the current study were about 0.5 °/ms (Fig. 4), similar to control values previously measured from video sequences for *Camponotus rufipes* (Gronenberg et al., 1997), indicating that the current mechanical probing method did not significantly affect the performance of the animals.

To generate forceful movements, all muscle fiber types are activated simultaneously. As mandibular force increases, the spike frequency increases proportionally in all muscle fiber types (Fig. 5). This does not indicate, however, that all muscle fibers contribute equally to the overall force of the mandible movement. Based on morphological and ultrastructural data (Gronenberg et al., 1997) and on our movement velocity measurements (Fig. 4), we conclude that muscle fibers with long sarcomeres generate slower yet more forceful movements. Therefore, slow muscle fibers contribute relatively more force to the mandible's overall force output. The maximum forces reached 41 mN in the current experiments (head width of *Camponotus rufipes* workers: 3.5–4.4 mm). While these are considerable forces, these values do not reflect the maximum forces possible. As we were interested in assessing the mandible movements, our mechanical loads (Fig. 1) were such that the ants were always able to readily close their mandibles. We therefore assume that the maximum forces that the ants can generate are considerably higher than is reflected by our values (large workers of *Camponotus rufipes* can penetrate human skin and draw blood even though their mandibles are relatively blunt).

In the current study we did not find any evidence suggesting sequential activation of different muscle fiber groups (Fig. 5). Rather, activity was found to occur simultaneously in anterior and posterior muscle fibers during complete mandible closing movements. Some of the slow movement episodes that we recorded were associated by activation of only one muscle fiber group (e.g., some data points in Fig. 5 reveal inactivity of fast fibers during these movement episodes), indicating that different muscle fibers can be controlled independently. We assume that such weak activation of particular muscle fibers supports the fine control of movements such as required for social interactions (under the recording conditions, our ants were not likely to engage in social behavior).

To summarize, we conclude that the ant mandible closer muscle is supplied by 10–12 motor neurons, which allows the ant to control the velocity and force of mandible movements. Some motor neurons supply the entire muscle whereas others control specific subsets of muscle fibers. Particularly fast movements result from activation of at least two fast motor neurons, which exclusively supply fast muscle fibers. When maximum force is required, all motor neurons are activated strongly and synchronously. In contrast, slow and delicate movements that require little yet precisely controlled force result from independent activation of only a few motor units.

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References

Akay, T., Bässler, U., Gerharz, P., Büschges, A., 2001. The role of sensory signals from the insect coxa-trochanteral joint in controlling motor activity of the femur–tibia joint. Journal of Neurophysiology 85, 594–604.

Allgäuer, C., Honegger, H.W., 1993. The antennal motor system of crickets: Modulation of muscle contractions by a common inhibitor; DUM neurons; and proctolin. Journal of Comparative Physiology A 173, 485–494.

Baines, R.A., Lange, A.B., Downer, R.G.H., 1990a. Proctolin in the innervation of the locust mandibular closer muscle modulates contractions through the elevation of inositol trisphosphate. Journal of Comparative Neurology 297, 479–486.

Baines, R.A., Tyrer, N.M., Downer, R.G.H., 1990b. Serotoninergic innervation of the locust mandibular closer muscle modulates contractions through the elevation of cyclic adenosine monophosphate. Journal of Comparative Neurology 294, 623–632.

Bartos, M., Allgäuer, C., Eckert, M., Honegger, H.W., 1994. The antennal motor system of crickets: Proctolin in slow and fast motoneurons as revealed by double labelling. European Journal of Neuroscience 6, 825–836.

Bässler, U., Büschges, A., 1998. Pattern generation for stick insect walking movements—multisensory control of a locomotor program. Brain Research Reviews 27, 65–88.

Bauer, C.K., Gewecke, M., 1991. Motoneuronal control of antennal muscles in Locusta migratoria. Journal of Insect Physiology 37, 551–562.

Burrows, M., 1996. The Neurobiology of an Insect Brain. Oxford University Press, Oxford, New York, Tokyo.

Clarac, F., Libersat, F., Pflüger, H.J., Rathmayer, W., 1987. Motor pattern analysis in the shore crab (*Carcinus maenas*) walking freely in water and on land. Journal of Experimental Biology 133, 395–414.

Costello, W.J., Govind, C.K., 1983. Contractile responses of single fibers in lobster claw closer muscles: Correlation with structure; histochemistry and innervation. Journal of Experimental Zoology 227, 381–393.

Griss, C., 1990. Mandibular motor neurons of the caterpillar of the hawk moth Manduca sexta. Journal of Comparative Neurology 296, 393–402.

Gronenberg, W., Tautz, J., Hölldobler, B., 1993. Fast trap jaws and giant neurons in the ant *Odontomachus*. Science 262, 561–563.

Gronenberg, W., 1995a. The fast mandible strike in the trap-jaw ant Odontomachus. I. Temporal properties and morphological characteristics. Journal of Comparative Physiology A 176, 391–398.

Gronenberg, W., 1995b. The fast mandible strike in the trap-jaw ant Odontomachus. II. Motor control. Journal of Comparative Physiology A 176, 399–408.

Gronenberg, W., Ehmer, B., 1996. The mandible mechanism of the ant genus Anochetus (Hymenoptera Formicidae) and the possible evolution of trap-jaws. Zoology 99, 153–162.

Gronenberg, W., Paul, J., Just, S., Hölldobler, B., 1997. Mandible muscle fibers in ants: Fast or powerful. Cell & Tissue Research 289, 347–361.

Gronenberg, W., Hölldobler, B., Alpert, G.D., 1998a. Jaws that snap: the mandible mechanism of the ant *Mystrium*. Journal of Insect Physiology 44, 241–253.

Gronenberg, W., Brandao, C.R.F., Dietz, B.H., Just, S., 1998b. Trap-jaws revisited: the mandible mechanism of the ant Acanthognathus. Physiological Entomology 23, 227–240.

Günzel, D., Galler, S., Rathmayer, W., 1993. Fibre heterogenity in the closer and opener muscles of crayfish walking legs. Journal of Experimental Biology 175, 267–281.

Hill, R.H., Govind, C.K., 1983. Fast and slow motoneurons with unique forms and activity patterns in lobster claws. Journal of Comparative Neurology 218, 327–333.

Hölldobler, B., Wilson, E.O., 1990. The Ants. Belknap Press of Harvard University Press, Cambridge, MA.

Homberg, U., Kingan, T., Hildebrand, J., 1987. Immunocytochemistry of GABA in the brain and suboesophageal ganglion of Manduca sexta. Cell & Tissue Research 248, 1–24.

Honegger, H.W., Brunninger, B., Bräunig, P., Elekes, K., 1990. GABA-like immunoreactivity in a common inhibitory neuron of the antennal motor system of crickets. Cell & Tissue Research 260, 349–354.

Hoyle, G., Burrows, M., 1973. Neural mechanisms underlying behavior in the locust Schistocerca gregaria I and II. Journal of Neurobiology 4, 3–67.

Hoyle, G., 1974. Neural control of skeletal muscle. In: Rockstein, M. (Ed.), The Physiology of Insecta, 2nd Ed., vol. IV. Academic Press, New York, London, pp. 175–236.

Hoyle, G., Dagan, D., Moberly, B., Colguhoun, W., 1974. Dorsal unpaired median insect neurons make neurosecretory endings on skeletal muscle. Journal of Experimental Biology 187, 159–165.

Janet, C., 1905. Anatomie de la tete du *Lasius niger*. Limoges, Paris.

Just, S., Gronenberg, W., 1998. The control of mandible movements in the ant Odontomachus. Journal of Insect Physiology 45, 231–240.

Kawasaki, F., Kita, H., 1995. Structure and innervation of longitudinal and transverse abdominal muscles of the cricket Gryllus bimaculatus. Journal of Comparative Neurology 352, 134–146.

Masuko, K., 1986. Motor innervation and proprioceptors of the mouthparts in the worker honeybee Apis mellifera: I. Mandibular nerve. Journal of Morphology 188, 51–68.

Müller, A.R., Wolf, H., Galler, S., Rathmayer, W., 1992. Correlation of electrophysiological; histochemical; and mechanical properties in fibres of the coxa rotator muscle of the locust *Locusta migratoria*. Journal of Comparative Physiology B 162, 5–15.

Orchard, I., Belanger, J.H., Lange, A.B., 1989. Proctolin: a review with emphasis on insects. Journal of Neurobiology 20, 470–496.

Ott, S.R., Jones, I.W., Burrows, M., Elphick, M.R., 2000. Sensory afferents and motor neurons as targets for nitric oxide in the locust. Journal of Comparative Neurology 422, 521–532.

Paul, J., Gronenberg, W., 1999. Optimizing force and velocity: Mandible muscle fibre attachments in ants. Journal of Experimental Biology 202, 797–808.

Paul, J., 2001. Review: Mandible movements in ants. Comparative Biochemistry and Physiology Part A 131, 7–20.

Pearson, K.G., Bergman, S.J., 1969. Common inhibitory motor neurons in insects. Journal of Experimental Biology 50, 445–471.

Rathmayer, W., 1996. Motorische Steuerung bei Invertebraten. In: Dudel, J., Menzel, R., Schmidt, R.F. (Eds.), Neurowissenschaft. Springer-Verlag, Berlin, Heidelberg, New York, pp. 167–190.

Rathmayer, W., Erxleben, C., 1983. Identified muscle fibers in a crab: I. Characteristics of excitatory and inhibitory neuromuscular transmission. Journal of Comparative Physiology 152, 411–420.

Rehder, V., 1989. Sensory pathways and motoneurons of the proboscis reflex in the suboesophageal ganglion of the honey bee. Journal of Comparative Neurology 279, 499–513.

Silverman, H., Costello, W.J., Mykles, D.L., 1987. Morphological fiber type correlates of physiological and biochemical properties in crustacean muscle. American Zoologist 27, 1011–1019.

Snodgrass, R.E., 1935. Principles of Insect Morpholgy. McGraw-Hill Book Company, New York.

Strausfeld, N.J., Seyan, H.S., Milde, J.J., 1987. The neck motor system of the fly Calliphora erythrocephala. Journal of Comparative Physiology A 160, 205–224.

Tyrer, N.M., Turner, J.D., Altman, J.S., 1984. Identifiable neurons in the locust central nervous system that react with antibodies to serotonin. Journal of Comparative Neurology 227, 313–330.

Wässle, H., Hausen, K., 1981. Extracellular Marking and Retrograde Labelling of Neurons. In: Heym, C., Forssmann, W.G. (Eds.), Techniques in Neuroanatomical Research. Springer-Verlag, Berlin, Heidelberg, New York, pp. 317–338.

Walther, C., 1980. Small motor axons in orthopteran insects. Journal of Experimental Biology 87, 99–119.

Wolf, H., 1990. On the function of locust flight steering muscle and its inhibitory innervation. Journal of Experimental Biology 150, 55–80.

Wolf, H., Burrows, M., 1995. Proprioceptive sensory neurons of a locust leg receive rhythmic presynaptic inhibition during walking. Journal of Neuroscience 15, 5623–5636.