Motor unit properties in the rabbit masseter muscle

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CHAPTER 7

MORPHOLOGY AND PHYSIOLOGY OF MASTICATORY MUSCLE MOTOR UNITS

Abstract - Motor unit territories in masticatory muscles appear to be smaller than territories in limb muscles and this would suggest a more localized organization of motor control in masticatory muscles. Motor unit cross-sectional areas show a wide range of values, which explains the large variability of motor unit force output. The proportion of motor unit muscle fibers containing more than one myosin heavy chain (MHC) isoform is considerably larger in masticatory muscles than in limb and trunk muscles. This explains the continuous range of contraction speeds found in masticatory muscle motor units. Hence, in masticatory muscles a finer gradation of force and contraction speeds is possible than in limb and in trunk muscles. The proportion of slow type motor units is relatively large in deep and anterior masticatory muscle regions, whereas more fast type units are more common in the superficial and posterior muscle regions. Muscle portions with a high proportion of slow type motor units are better equipped for a finer control of muscle force and a larger resistance to fatigue during chewing and biting than muscle portions with a high proportion of fast units. For the force modulation, masticatory muscles rely mostly on recruitment gradation at low force levels and on rate gradation at high force levels. Henneman's principle of an orderly recruitment of motor units has also been reported for various masticatory muscles. The presence of localized motor unit territories and task specific motor unit activity permits differential control of separate muscle portions. This gives the masticatory muscles the capacity of producing a large diversity of mechanical actions. In this review the properties of masticatory muscle motor units are discussed.
Introduction

During mastication and biting, the masticatory muscles generate forces that are responsible for the movements and deformations of the jaw and for the production of forces at the teeth and temporomandibular joints. For an extensive review of various aspects of the functional anatomy of the masticatory muscles and their control the reader is referred to a number of recent publications (Lund, 1991; Miller, 1991; Mao et al., 1992; Hannam and McMillan, 1994). The motor unit can be considered as the basic unit of motor activity, since it is the smallest unit that can be recruited and controlled by the central nervous system. It consists of an α motoneuron and the set of muscle fibers innervated by this neuron. Each muscle fiber is innervated by only one motoneuron and each motoneuron may innervate tens to thousands of muscle fibers. Most muscles possess several hundred motor units. When a motoneuron is excited to discharge action potentials, the muscle fibers of the motor unit are activated. As a result, these fibers start contracting and produce a force. Motor units show a large variability in morphological and physiological characteristics which result in a wide range of properties with respect to, for example, force output, contraction speed, and fatigability.

There is a large body of knowledge on the properties of motor units in limb and trunk muscles (see for recent reviews: Miles, 1994; Enoka, 1995; Sargeant and Jones, 1995; McComas, 1998). However, such knowledge regarding masticatory muscle motor units is relatively sparse and it is not unreasonable to expect that they have different properties compared to trunk and limb muscle motor units. The masticatory muscles are innervated by cranial nerves, whereas the limb and trunk muscles are innervated by spinal nerves. The cell bodies of the motoneurons of the masticatory muscles are located in the brain stem. A peculiar feature is that afferent feedback from muscle spindles in the jaw opening muscles is scarce or lacking (Van Willigen et al., 1993). Masticatory muscle fibers have a number of characteristics that differ from limb and trunk muscles. They have considerably smaller cross-sectional areas than limb muscle fibers. Also, the type II fibers have smaller cross-sectional areas than type I fibers (Eriksson and Thornell, 1983; Korfage and Van Eijden, 1999, 2000), whereas the reverse is true in limb and trunk muscles (Polgar et al., 1973). In masticatory muscles, grouping of muscle fibers of the same type can be found (human: Eriksson and Thornell, 1983; rabbit: Bredman et al., 1990), in
contrast to the mosaic pattern in limb and trunk muscles, where fiber-type grouping is considered as a pathological sign of denervation and re-innervation. With respect to the phenotype of their contractile proteins, masticatory muscle fibers express myosin isoforms that are normally not expressed in adult trunk and limb muscles (Butler-Browne et al., 1988; Bredman et al., 1991a; d’Albis et al., 1991; Stål et al., 1994). In addition, in masticatory muscles, a relatively large number of fibers express more than one myosin isoform (Stål et al., 1994; Korfage and Van Eijden, 1999, 2000), while recent results indicate that in trunk and limb muscles this phenomenon is primarily observed in elderly subjects (Andersen et al., 1999).

One of the main reasons for the relative scarcity of information on masticatory motor units is that, in experimental animals, the motor roots of masticatory muscles are less accessible for fiber splitting and subsequent electrical stimulation than those of limb and trunk muscles. The only report on a method of fiber stimulation was by Druzinsky (1996) who stimulated single motor units in the rat masseter by impaling axons in the mandibular nerve. Just recently, extracellular stimulation of motoneurons in the rabbit trigeminal motor nucleus enabled the documentation of a large number of masticatory motor unit properties (Weijs et al., 1993; Kwa et al., 1995a,b; Kwa and Weijs, 1999; Kwa and Van Eijden, 2000; Turkawski et al., 1996, 1998; Turkawski and Van Eijden, 2000a,b,c). In the human external stimulation of masticatory motor units through the skin, like, for example, stimulation of the median nerve to stimulate motor units of the thenar muscles (Chan et al., 1998), is not possible. Alternatively, masticatory motor unit twitches have been recorded in bite force registrations by using the so-called spike-triggered averaging technique (for example, see Nordstrom et al., 1989; McMillan et al., 1990). However, its applicability to obtain motor unit properties is very limited.

The present review examines current knowledge of morphological and physiological properties of masticatory motor units. Special emphasis will be given to the variability of masticatory motor unit properties, the determinants of this variability and their implications for masticatory muscle functioning. The review is divided into three parts. In part 1, motor unit morphology, including the size and shape of motor unit territories and the number of muscle fibers per motor unit, is evaluated. In part 2, motor unit contractile properties, such as motor unit force, speed of contraction, and fatigability are reviewed. Also, the relationship between biochemical properties of muscle fibers and the motor unit’s contractile properties will be summarized.
Finally, in part 3, the mechanisms recruitment and rate gradation which are applied by the central nervous system to modulate muscle force are discussed.

**Motor unit morphology**

**Size and shape of motor unit territory**

In animal muscles, motor unit territories can be mapped with the glycogen depletion method that was originally described by Edström and Kugelberg (1968). In this method, a neuron is stimulated until the muscle fibers it innervates are depleted of glycogen. These depleted fibers can then be visualized in a muscle histological section where they are the only ones that are not stained by the periodic acid Schiff (PAS) method (Fig. 1). With three-dimensional reconstructions from subsequent muscle sections, the number and spatial distribution of the motor unit fibers and the three-dimensional size, position, and orientation of the motor unit territory and its relationship to tendon plates within the muscle can be ascertained.

Glycogen depletion studies have shown that the muscle fibers of motor units are intermingled with each other and are restricted to a particular region of the muscle, i.e., the motor unit territory. Within the territory of a motor unit, fibers of 15-30 other motor units can be found (Buchthal and Schmalbruch, 1980). The distance between fibers of a particular motor unit varies from zero (fibers in contact) to hundreds of micrometers. The fibers of a motor unit are primarily arranged in parallel and not in series. Motor unit territories are often elliptically shaped and the relative territory occupied by a motor unit varies. In cat hindlimb muscles (Bodine-Fowler et al., 1990), for example, the cross-sectional area of the motor unit territories ranges between 8-76% of the whole muscle cross-sectional area.

As most fibers of a motor unit are surrounded by fibers of other motor units, tension produced by an active motor unit is probably not only directly transmitted to tendons and aponeuroses, but also indirectly through these surrounding passive or active fibers (Trotter, 1993; Huijing et al., 1998). Hence, it is likely that muscle fibers might possess shear properties that permit them to transmit tension to neighboring muscle fibers.
While in human muscles, motor unit territories cannot be assessed by the glycogen depletion technique, Stålberg and Antoni (1980) developed the technique of electrophysiological cross-sectional scanning of motor units (scanning EMG) in human muscles. In this technique, an electrode is moved through the territory of an active motor unit. The length of the path over which motor unit activity is registered is used as an estimation of motor unit width. Using scanning EMG, Stålberg and Eriksson (1987) and McMillan and Hannam (1991) estimated motor unit territory width in the human masseter muscle. In these studies, motor units were recruited at low to moderate clench forces and therefore most recordings were likely to originate from low threshold slow type motor units. Since these units are preferentially located in the deep part of the masseter muscle (see below), the motor unit sample was probably from the deep masseter. Stålberg and Eriksson (1987) found a medio-lateral motor unit diameter of 3.7 ± 0.6 mm (mean ± SD; range: 0.6-12.5 mm; n=32) and McMillan and Hannam (1991) found a mean medio-lateral and antero-posterior diameter of 3.2 ± 2.3 mm (range: 0.1-10.0 mm; n=32) and 6.1 ± 4.0 mm (range: 0.3-

Figure 1.
Photomicrograph of a cryosection of rabbit masseter muscle showing a territory of a motor unit. Motor unit fibers are not stained by the PAS method, because they are glycogen depleted after exhaustive stimulation of the motoneuron. At the top and bottom, horizontally oriented tendon sheets can be seen.
19 mm), respectively. Tonndorf and Hannam (1994) reported comparable sizes (n=162) for the human masseter. Since the masseter muscle depth and width is about 10-15 mm and 40 mm, respectively, these data indicate that the territories occupy a limited portion (on average less than about 5%) of the muscle's cross-sectional area. Because of their larger antero-posterior diameter, the territories can be considered to have medio-laterally flattened, elliptical cross sections. They are considered to be arranged in layers throughout the muscle (McMillan and Hannam, 1991). The three-dimensional territories are at least, approximately, three times longer than wide, as the masseter muscle fiber length is on average 21.3 ± 2.9 mm (Van Eijden et al., 1997). By combining scanning EMG and high resolution magnetic resonance imaging (MRI), Tonndorf and Hannam (1994) were able to determine motor unit location relative to intramuscular tendon sheets. They found that most territories were located within discrete tendon-bounded compartments, while only 10% extended across tendons. The latter motor units were relatively large and did not appear specific to any muscle region. The reported human masseter motor unit territories were small compared to those in human arm and leg muscles (Stålberg et al., 1986).

Using the glycogen depletion method, Herring and co-workers mapped masseter muscle motor unit territories in infant and adult miniature pigs (Herring et al., 1989, 1991). In both piglets and older animals, the territories were generally small and restricted, taking up no more than approximately 5-10% of the total muscle volume. These authors stimulated nerve filaments containing 1 to 5 axons.

### Table 1. Morphological characteristics of rabbit masseter motor units

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>mean</th>
<th>SD</th>
<th>min</th>
<th>max</th>
</tr>
</thead>
<tbody>
<tr>
<td>cross-sectional territory area (mm²)</td>
<td>7.9</td>
<td>3.4</td>
<td>1.1</td>
<td>19.6</td>
</tr>
<tr>
<td>length (mm)</td>
<td>9.9</td>
<td>4.1</td>
<td>5</td>
<td>23</td>
</tr>
<tr>
<td>innervation ratio</td>
<td>241</td>
<td>158</td>
<td>40</td>
<td>720</td>
</tr>
<tr>
<td>% motor unit fibers within area</td>
<td>10</td>
<td>5</td>
<td>1</td>
<td>20</td>
</tr>
<tr>
<td>physiological cross-sectional area (mm²)</td>
<td>0.9</td>
<td>0.7</td>
<td>0.1</td>
<td>3.0</td>
</tr>
</tbody>
</table>

*Data from Kwa et al. (1995a)*
Therefore, their experiments primarily involved multiple motor units. Weijs and coworkers (Weijs et al., 1993; Kwa et al., 1995a) were able to induce glycogen depletion in single motor units (n=32) of the rabbit masseter muscle by extracellular stimulation of motoneurons in the trigeminal motor nucleus. Because the extracellular stimulation technique preferentially stimulates large motoneurons, slow type motor units with small motoneurons can not easily be recruited. Therefore, fast type motor units were preferentially excited. Slow type fibers, however, are present in only small amounts (<2%) in rabbit masseter muscle (Bredman et al., 1992; Sciote and Kentish, 1996). Territory cross-sectional areas in the rabbit masseter were relatively small (Table 1). The mean cross-sectional area was 7.9 mm² in a total mid-belly muscle cross-section of about 200 mm². Hence, although in the rabbit masseter, motor unit territories are smaller than in the human masseter, they occupy, on average, about a similar percentage (4%) of the muscle's mid-belly cross-sectional area. Territory areas in the rabbit masseter appeared to vary considerably in size (Table 1) and had a large diversity in shapes, both regular and irregular, with the regular cross sections varying from ellipsoid to round. Three-dimensional reconstructions revealed that the shape of the territories was elongated along the mean direction of the muscle fibers. The length of the motor units (mean length: 9.9 mm) exceeded the length of the muscle fibers (6-8 mm, Langenbach and Weijs, 1990), which indicates the tendency for motor unit fibers to extend their area of attachment to an aponeurosis in the direction of the long axis of the muscle, rather than sideways (Weijs et al., 1993). Rabbit masseter motor unit territories were usually restricted to single anatomical compartments, i.e., the motor unit fibers run from one side of the aponeurosis of origin to the facing side of the parallel insertion aponeurosis. Restriction of these motor unit territories to small muscle sub-volumes was mechanically confirmed by Turkawski et al. (1998). They attached a two-component force transducer to the zygomatic arch of the rabbit and registered both magnitude, position and direction of masseter motor unit force after extracellular motoneuron stimulation. Their results showed that the lines of action of motor units within the masseter muscle have indeed a large variety of positions and directions and that the variation of action lines was almost as large as the range of fiber directions inside the muscle.

The results described above indicate that in the human and animal masseter, the motor unit territories are small and restricted to specific areas. Such an
organization permits differential control of separate muscle portions. It permits the production of internal force vectors with different directions and magnitudes in muscles with broad attachment areas and heterogeneous skeletal lever arms resulting in differential mechanical actions (Van Eijden et al., 1988; Van der Helm and Veenbaas, 1991; Van Eijden and Koolstra, 1998). Such a differential control has indeed been established for a number of masticatory muscles in the human (Belser and Hannam, 1986; Tonndorf et al., 1989; Blanksma and Van Eijden, 1990; Blanksma et al., 1992; Van Eijden et al., 1993; Murray et al., 1999), rabbit (Weij and Dantuma, 1981) and pig (Herring et al., 1979). It should be noted that the results of Langenbach et al. (1992) in the rabbit, and of Herring and Wineski (1986) in the pig indicate that contraction patterns become more heterogeneous with age. This might imply that infant muscles are less capable of differential contraction, which might be due to larger and overlapping motor units in younger muscles, which in turn might be the result of polyneuronal innervation. However, glycogen depletion experiments in pig masseter demonstrated that motor unit territories were already very restricted in younger animals (Herring et al., 1991).

Interestingly, motor unit territorial areas in the masseter muscle occupied only about 5% of the total muscle cross-sectional area and this is less than the motor unit territories reported in various limb muscles where motor unit territories occupy 10-50% of the muscle’s cross section (Edström and Kugelberg, 1968; Burke and Tsairis, 1973; Burke et al., 1974; English and Weeks, 1984; Bodine et al., 1987). This suggests a more localized organization of motor control in masticatory muscles.

**Number of muscle fibers per motor unit**

The number of muscle fibers innervated by one motoneuron, i.e., the innervation ratio, is an important factor in determining the motor unit force. The maximum force a motor unit can produce is proportional to its physiological cross-sectional area. This is the sum of transverse cross-sectional areas of all its muscle fibers. The larger the innervation ratio, the larger the motor unit force. The innervation ratio differs across muscles. Small muscles, for example, eye muscles, intrinsic hand muscles, have lower innervation ratios (dozens) than large muscles (thousands), for example, quadriceps and gastrocnemius muscles, and this enables smaller muscles to exert muscle force more finely than larger muscles. The innervation ratio also varies within
a particular muscle and varies, among other considerations, with muscle fiber characteristics. For example, in the tibialis anterior of the cat (Bodine et al., 1987) and medial gastrocnemius muscle of the rat (Kanda and Hashizume, 1992) the innervation ratio of motor units consisting of slow type fibers is lower than that of motor units consisting of fast type fibers. Hence, slow type motor units produce less force than fast type units.

In human muscles, the innervation ratio can be estimated from autopsy material. The number of motor units in a particular muscle can be assessed by estimating the number of axons of α motoneurons in a section of the nerve innervating the muscle. The mean innervation ratio is calculated by dividing the total number of muscle fibers by the total number of α motoneurons. It should, however, be noted that there are some uncertainties with respect to the estimation of the number of α motoneurons (Enoka, 1995), such as the errors associated with distinguishing between small- and large-diameter myelinated axons and between afferent and efferent axons. Carlsson (1958) estimated 1,452 and 1,331 motor units in the human masseter and temporalis muscle, respectively, of one male cadaver. The estimated number of muscle fibers in these muscles was 929,000 and 1,247,000, respectively. Therefore, the calculated innervation ratios were 640 for the masseter and 936 for the temporalis. The estimated average motor unit physiological cross-sectional area was 0.22 mm$^2$ for the masseter and 0.29 mm$^2$ for the temporalis. Assuming an intrinsic tension of 30 N/cm$^2$ for human masticatory muscles (Weijs and Hillen, 1985), the corresponding motor unit force would be 66 mN for the masseter muscle and 81 mN for the temporalis, which is within the range of tetanic motor unit forces reported by Turkowski et al. (1998) for the rabbit masseter muscle.

Using the glycogen depletion method, innervation ratios in the pig masseter (Herring et al., 1989) were found to vary between 5 and 400, and in the rabbit masseter (Kwa et al., 1995a) between 40 and 720 (Table 1). In the rabbit masseter, the number of motor unit fibers expressed as a percentage of the total number of fibers present within a motor unit area, i.e., the relative density, was on average 10%. This density showed a large variability. Using single fiber EMG and macro EMG, Stålborg et al. (1986) examined the size and fiber distribution of motor units in human masseter and temporalis muscles. Their results indicate that the fiber density in the motor units of the temporalis was larger than in the masseter muscle. For the
rabbit masseter, Kwa et al. (1995a) found no correlation between innervation ratio and the size of the territory but a high positive correlation between innervation ratio and relative density. Hence, in the rabbit masseter an increase in fiber number is not associated with an increase of territorial area, but with a larger concentration of motor unit fibers per mm$^2$. Limb muscles of an animal of comparable size (cat) have smaller relative densities (Burke and Tsairis, 1973, Burke et al., 1974: 1-5%; Armstrong et al., 1988: 5-9%), because motor unit territories of the rabbit masseter are smaller and have the same range of innervation ratios. The physiological cross-sectional area of the rabbit masseter motor units was reported to vary between 0.1 and 3.0 mm$^2$, pointing to a large variability of possible motor unit forces (Kwa et al., 1995a). Indeed, such a large variability has been demonstrated experimentally (Turkawski et al., 1998; Turkawski and Van Eijden, 2000b). It can be concluded that in rabbit masseter muscle, an increase in motor unit force may not affect the capability of differential control of separate muscle portions, because a larger number of muscle fibers per motor unit is accompanied by an increase of fiber density and not by an increase of territorial area.

Motor unit contractile properties

Characterization of contractile properties

As indicated above, motor units can be stimulated electrically by using stimulating electrodes. The resulting contraction of the unit is generally measured under isometric conditions using a force transducer. After a single activation pulse, a twitch contraction in all the fibers of the unit is generated. The twitch of a motor unit is a combination of all single fiber twitches (Fig. 2). The contractile speed of the unit is generally defined by the so-called twitch contraction time, which is the time to peak force. The contractile twitch force of the unit is defined by the amplitude of the twitch. Twitch force magnitude depends on the innervation ratio, the cross-sectional area of the muscle fibers, and the specific tension of the muscle fibers, i.e., force per unit of cross-sectional area. When a motoneuron is activated by a train of pulses, a series of similar twitches will be produced. At a sufficiently high stimulus rate, the successive twitches will summate and fuse, resulting in a larger force than that of the
individual twitches. The stimulus frequency at which complete fusion occurs is called the fusion frequency. A further increase in the stimulus rate above the fusion frequency causes a further increase of force until maximum tetanic force is reached.

Fatigue can be defined as a decline in force during stimulation. A motor unit is fatigue resistant if its force shows a relatively small or no decline, whereas a fatigable unit shows a relatively large decline. The onset of fatigue is found to be associated with, for example, fiber type, stimulation history, and stimulation intensity (Burke, 1967).

![Diagram of Twitch Registration](image)

**Figure 2.**
Twitch registration of a relatively slow and fast motor unit of the rabbit masseter muscle. TCT: twitch contraction time, TF: twitch force. For the slow unit TCT=34 ms and TF=25 mN, for the fast unit TCT=16 ms and TF=93 mN.
Based on their contraction velocity and fatigability, motor units have been classified (Burke, 1967; Burke et al., 1973, 1974) into different types (Table 2): S (slow, fatigue-resistant), FR (fast, fatigue-resistant), Fint (fast, intermediate fatigable), and FF (fast, fatigable). This classification is primarily based on cat hindlimb motor units. Fast units have twitch contraction times of less than 40 ms (Fig. 2), whereas slow units have longer twitch contraction times. Unfused tetani are produced if a motor unit is stimulated at intervals slightly longer than 1.25 times its twitch contraction time. After a while the force may decrease. This phenomenon, called the sag property, is considered a reliable way to distinguish between fast and slow motor units (Burke, 1967; Burke et al., 1973). Fast units show sag, whereas slow units do not. The magnitude of the maximum tetanic force output is correlated to the motor unit type: it decreases in the order of FF-Fint-FR-S. The fatigability of motor units is defined by the so-called fatigue index (Burke et al., 1973, 1974). It is the ratio of tetanic force produced after a stimulus regimen of 2 minutes (120 stimulus trains, 1 stimulus train/s, 13 pulses/train at 40 Hz) to the tetanic force produced at the first stimulus train. Fatigable (F) units have fatigue indices of less than 0.25, intermediate fatigable (int) units have fatigue indices between 0.25 and 0.75, and fatigue resistant (R) units have fatigue indices larger than 0.75.

**Twitch and tetanus force**

Information on twitches of motor units in human and monkey masticatory muscles has been obtained from the spike-triggered averaging technique (Goldberg and

| Table 2. Physiological classification of motor units$^a$ |
|-----------------|-----------------|------------|--------------|
| motor unit type | twitch contraction time (ms) | sag | fatigue index |
| slow (S)        | > 40             | no      | > 0.75       |
| fast fatigue resistant (FR) | < 40             | yes      | > 0.75       |
| fast intermediate fatigable (Fint) | < 40             | yes      | 0.25 - 0.75   |
| fast fatigable (FF) | < 40             | yes      | < 0.25       |

$^a$ based on cat hindlimb muscles (see Burke, 1967; Burke et al., 1973, 1974)
Table 3. Twitch contraction time (TCT) and twitch force of masticatory muscle motor units determined with spike-triggered averaging

<table>
<thead>
<tr>
<th></th>
<th>number of motor units</th>
<th>TCT (ms)</th>
<th>twitch force (mN)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>min</td>
<td>max</td>
</tr>
<tr>
<td>Goldberg and Derfler (1977)</td>
<td>29</td>
<td>38</td>
<td>69</td>
</tr>
<tr>
<td>Yemm (1977)</td>
<td>149</td>
<td>24</td>
<td>91</td>
</tr>
<tr>
<td>Nordstrom and Miles (1990)</td>
<td>37</td>
<td>20</td>
<td>72</td>
</tr>
<tr>
<td>McMillan et al. (1990)</td>
<td>32</td>
<td>25</td>
<td>67</td>
</tr>
<tr>
<td>Clark et al. (1978)</td>
<td>24</td>
<td>17</td>
<td>31</td>
</tr>
</tbody>
</table>

*Human masseter, † human masseter and temporalis, ‡ monkey temporalis

Derfler, 1977; Yemm, 1977; Clark et al., 1978; Nordstrom and Miles, 1990; McMillan et al., 1990). With this technique, a subject is instructed to produce a bite force. Simultaneously, the activity of a single motor unit is recorded from an electrode inserted into the muscle. Using feedback, the subject is capable of maintaining a low discharge rate of the unit and its twitch force can be extracted from the total bite force signal. The firing rate is usually between 7 and 10 Hz, which implies that the twitches are in fact partially fused (Nordstrom et al., 1989). The twitch profiles measured with this method are biased by a number of factors, including firing rate, coactivation of muscles and muscle structure (see for an overview: McMillan et al., 1990). Furthermore, bite forces located at the teeth were measured. Because the mandible acts as a lever, twitch forces are considerably (up to 50%) smaller than those produced in the muscle under consideration and variation of twitch force is partly due to variation in motor unit position with respect to tooth location. In addition, bite force transducers capable of registering force in only one direction have been used which may also contribute to the underestimation of forces (Van Eijden et al., 1990).

Twitch contraction time and twitch force amplitude for human and monkey masticatory motor units (Table 3) have been reported to vary considerably (Goldberg and Derfler, 1977; Yemm, 1977; Clark et al., 1978; McMillan et al., 1990; Nordstrom and Miles, 1990). These studies point to a continuous distribution of twitch
contraction times, without separate groups of slow and fast motor units. In contrast to the results from animal studies, a poor or no correlation between twitch amplitude and twitch contraction time has been reported (Yemm, 1977; Goldberg and Derfler, 1977; Nordstrom and Miles, 1990).

Information on the contractile properties of motor units of masticatory muscles in animals is scarce. As described above, characteristics of rabbit masseter motor units have been examined by extracellular stimulation of motoneurons in the brainstem (Weijs et al., 1993; Kwa et al., 1995a,b; Kwa and Weijs, 1999; Kwa and Van Eijden, 2000; Turkawski et al., 1996, 1998; Turkawski and Van Eijden, 2000a,b,c). In these studies, motor unit forces were registered indirectly, via a one-component transducer attached to the teeth (Weijs et al., 1993; Kwa et al., 1995a,b; Kwa and Weijs, 1999; Kwa and Van Eijden, 2000), or directly, via a two-component force transducer attached to the zygomatic arch (Turkawski et al., 1996, 1998; Turkawski and Van Eijden 2000a,b,c). A major advantage of the latter method is that the position and direction of motor unit force in the masseter muscle could be registered and that no transformation was needed from the force registered at the teeth.

Twitch contraction time and twitch peak force for 249 masseter motor units in 41 rabbits was registered by Kwa et al. (1995b) and for 78 motor units in 8 rabbits by

| Table 4. Contraction characteristics of rabbit masseter motor units |
|----------------------|----------------------|----------------------|
|                      | Kwa et al. | Turkawski et al. |
|                      | mean | SD | min | max | mean | SD | min | max |
| twitch peak force (mN) | 36.8 | 49.2 | 7.2 | 248.8 | 21.9 | 26.2 | 2 | 100 |
| twitch contraction time (ms) | 21.0 | 4.9 | 13 | 32 | 25.3 | 5.5 | 16 | 42 |
| tetanus force (mN) | 87.5 | 66.8 | 5 | 290 |
| twitch-tetanus ratio | 0.38 | 0.15 | 0.19 | 0.84 | 0.30 | 0.16 | 0.10 | 0.57 |
| sag ratio | 0.77 | 0.13 | 0.49 | 0.94 |
| fatigue index | 1.04 | 0.25 | 0.25 | 1.30 |
| fusion frequency (Hz) | 48 | 8 | 34 | 64 |

Data from: a Kwa et al. (1995b) b Kwa and Weijs (1999) c Turkawski et al. (1998)
Turkawski et al. (1998). In the latter study, tetanus force was also registered. These studies indicate that rabbit masseter motor units are very fast (Table 4). Twitch contraction time showed a continuous range between 13 and 32 ms (Kwa et al., 1995b) and between 16 and 42 ms (Turkawski et al., 1998). This range is relatively small and indicates that the masseter muscle of the rabbit contains only fast motor units. In the similarly sized cat, twitch contraction time in limb muscles has been reported to range between 15-20 ms for the fastest units and between 40-110 ms for the slowest units (Burke et al., 1973, 1974; Bodine et al., 1987; Lev-Tov et al., 1988). Twitch contraction time (mean range: 27-31 ms) determined after nerve stimulation for several jaw closing and opening muscles in the cat also suggests that the masticatory muscles are relatively fast (Mackenna and Türker, 1978). A comparable result was found by Taylor et al. (1973), who directly stimulated small strips of masseter (13.1 ± 2.3 ms, n=18) and temporalis muscle (11.4 ± 2.1 ms, n=21) of the cat. It should be noted that twitch contraction time increases with motor unit length. Turkawski and Van Eijden (2000b) demonstrated for rabbit masseter motor units that during a jaw opening from 0° to 21° twitch contraction time increased by about 30%.

Twitch force in the rabbit masseter also showed a large variability and ranged between 5 and 250 mN (Kwa et al., 1995b) and between 2 and 100 mN (Turkawski et al., 1998). The majority of motor units (>80%), however, generated forces smaller than 35 mN. There was a small but significant negative correlation between twitch force and twitch contraction time. Hence, the units that produced larger forces were faster and, therefore, it seems safe to assume that the physiological cross-section of faster motor units is larger than that of slower units, due to a larger innervation ratio and/or larger fiber diameters. Interestingly, different motor unit types were found to be distributed heterogeneously throughout the masseter (Turkawski et al., 1998, 2000b). The slowest contractions were produced by motor units in the anterior masseter and the fastest contractions by units in the posterior deep masseter. In addition, motor units in the anterior masseter showed more variability in force output compared to the posterior masseter and motor units in the deep masseter produced considerably less force (average: 25-50 mN) than motor units in the superficial masseter (average: 45-50mN). Antero-posterior differences in contraction times are supported by histochemical studies (Bredman et al., 1990) which reveal that slower
muscle fibers predominate in deep and anterior regions, whereas faster fibers predominate in more superficial and posterior regions (see below).

The force produced by masticatory motor units also depends on their length. During jaw movements they will undergo length changes and, because of the length-force relationship of the sarcomeres, their force output will vary. The average jaw angle at which masseter motor units produce maximum force is 12° and force output is relatively low (20-60% of maximum force) at occlusion and relatively high (60-100% of maximum force) at a jaw gape of 21° (Turkawski and Van Eijden, 2000b).

Tetanus force is considerably larger than twitch force. Turkawski et al. (1998) found a mean twitch force of 21.9 mN and a mean tetanus force of 87.5 mN, and a mean twitch-tetanus ratio of 0.30. Kwa and Weijis (1999) found a mean twitch-tetanus ratio of 0.38. From the results of Taylor et al. (1973) twitch-tetanus ratios can be calculated to be 0.13 for the cat masseter and 0.11 for the cat temporalis. When compared to these ratios and that of the cat hindlimb muscles (range: 0.04 - 0.17), the twitch-tetanus ratio for rabbit masseter motor units is relatively high (Fleshman et al., 1981; Kernell et al., 1983a; Bodine et al., 1987; Robinson et al., 1991). This finding has been explained by the larger capacity for serial elasticity in hindlimb muscles (Kwa and Weijis, 1999).

From the results described above it can be concluded that data on twitches of human masticatory motor units should be interpreted with care, because the spike-triggered averaging technique has a number of limitations. The large variability of motor unit twitch and tetanic force seems, primarily, to be due to the variability of motor unit cross-sectional area, as has been demonstrated for the rabbit masseter muscle.
Figure 3.
Light micrographs of six consecutive sections of the human digastric muscle (posterior belly) incubated with monoclonal antibodies against myosin heavy chain (MHC). Examples of fibers expressing different MHC isoforms: 1 = MHC-I, 2 = MHC-IIA, 3 = MHC-I/α, 4 = MHC-I/IIA/IIX/fetal, 5 = MHC-IIX; bar: 50 μm.
**Speed of contraction and myosin type**

The speed of contraction of motor units is largely dependent on the heavy chain of the myosin proteins (myosin heavy chain, MHC). Immunohistochemical techniques have revealed that in adult mammalian limb muscles at least four different isoforms of MHC are expressed: MHC-I, MHC-IIA, MHC-IID (or MHC-IIX) and MHC-IIB (Schiaffino and Reggiani, 1994). In the aforementioned order, these four isoforms demonstrate an increasing rate of ATP consumption and speed of contraction. Fibers containing more than one type of MHC (for example I/IIA, or IIA/IID, or IID/IIB) have intermediate contraction speeds (Larsson and Moss, 1993). The fiber specific tension does not seem to depend on MHC isoform type (Schiaffino and Reggiani, 1996). In adult masticatory muscles, two other MHC isoforms have been found: MHC-fetal (d'Albis et al., 1991) and MHC-cardiac α, which is identical to the cardiac specific MHC found in adult myocardium (Bredman et al., 1991a). It is not known why the masticatory muscles have so many fibers expressing MHC-fetal or MHC-cardiac α. It is possible that the expression of these MHCs is determined by the connective tissue derived from neural crest cells (Bredman et al., 1991a). The MHC composition of muscle fibers can be mapped immunohistochemically with monoclonal antibodies against different MHC-isoforms (Fig. 3). Correlations between contractile properties of motor units and MHC content of motor unit fibers can be established by first classifying a motor unit physiologically and then classifying glycogen depleted fibers on the basis of their immunoreactivity for MHC isoforms.

Differences in contraction velocity of muscle fibers can also be assessed using the traditional ATPase staining method. ATPase classified fiber types I, IIA and IIB are, in general, found to correlate well with the immunohistochemically classified MHC-I, MHC-IIA and MHC-IIB fiber types (Staron and Pette, 1986). However, the ATPase technique cannot discriminate between MHC-IID and MHC-IIB (Schiaffino et al., 1989). Therefore, in a number of studies, masticatory muscle fibers have erroneously been classified as being type IIB instead of type IID (Eriksson and Thornell, 1983; Bredman et al., 1990). Furthermore, type IM and IIC fibers, showing intermediate ATPase reactions have been described (Brooke and Kaiser, 1970; Ringqvist, 1974; Eriksson and Thornell, 1983). These intermediate fiber types appear to contain two types of MHC (Thornell et al., 1984; Bredman et al., 1992). Thus, the ATPase method has it limits because it is unable to discriminate
between all MHC isoforms, including MHC-fetal and MHC-cardiac α, and it cannot distinguish the MHC isoforms that can appear in any given fiber.

In the last decade, the MHC content of motor unit fibers has been correlated with their physiological properties. S units were associated with MHC-I, FR units with MHC-IIA, and FF units with MHC-IIIB (Larsson and Moss, 1993). Motor units are not necessarily homogeneous with respect to the MHC content of their muscle fibers. A motor unit may consist of fibers with different types of MHC (rat lumbrical: Gates et al., 1991; rat tibialis anterior: Larsson et al., 1991). As stated previously, in one fiber more than one MHC type (e.g., I/IIA or IIA/IID) may be found (Aigner et al., 1993; Staron and Pette, 1993). These so-called hybrid fibers show intermediate contraction speeds. It has been suggested that these hybrid fibers indicate fiber type transformation, as a result of, for example, an increase or decrease of activity (for review, see Pette and Vrbová, 1985). The following transition pathway of MHC isoforms has been suggested: I ↔ I/IIA ↔ IIA ↔ IIA/IID ↔ IID/IIB ↔ IIB (Gorza, 1990; DeNardi et al., 1993). The proportion of hybrid fibers is considerably larger in masticatory muscles than in limb and trunk muscles (Butler-Browne et al., 1988; Stål et al., 1994; Korfage and Van Eijden, 1999, 2000). This parallels the finding that masticatory muscles contain a relatively large number of ATPase intermediate (IM) fibers (Ringqvist, 1974; Eriksson and Thornell, 1983; Rowlerson et al., 1983). The relatively large number of hybrid fibers confirms the continuous range of contraction speeds found in masticatory muscle motor units and facilitates a fine gradation of muscle contraction speeds.

A strong correlation between MHC isoform content and twitch contraction time has been demonstrated for motor units in the masseter muscle of the rabbit (Kwa et al., 1995b). In this latter investigation one motor unit was physiologically classified as FF unit and the remaining units as FR. No MHC-IIIB was found. The fibers of the FF unit contained MHC-IID. Of the FR units, five were found to have a single MHC (3 MHC-IIA and 2 MHC-α). Eighteen motor units were found to be heterogeneous: in three motor units all fibers contained one combination of two types of MHC (1 MHC-IIA/IID, 1 MHC-IIA/α, 1 MHC-α/I), and in fifteen motor units, two types of MHC were spread unevenly over the constituting fibers. No motor units were found with fibers containing pure MHC-I, which is in agreement with Bredman et al. (1992) and Sciote and Kentish (1996) who found that MHC-I fibers are relatively rare in the adult rabbit masseter muscle. MHC composition of the motor
units was plotted against twitch contraction time (Kwa et al., 1995b). It appeared that the continuous range of contraction speeds of the motor units matched the continuous spectrum of MHC mixtures and that the contraction speed of fibers increased in the order of I-α-IIA-IID. The position of MHC-cardiac α in this range, between MHC-I and MHC-II types, was also found by Sciote and Kentish (1996) using the slack-test method on skinned rabbit masseter muscle fibers. Because of these mixtures of MHC isoforms, classification of motor unit types can be expected to be less distinct in masticatory muscles than in limb and trunk muscles. This may imply that in masticatory muscles, a finer gradation of force and movement is possible than in trunk and limb muscles. As Kwa et al. (1995b) did not have antibodies against MHC-fetal at their disposal, the position of this MHC in the contraction speed sequence is not known.

Fiber type composition of a particular masticatory muscle may vary considerably among individuals. These differences might be related to differences in, for example, biopsy location, age, or gender (English et al., 1998; Tuxen et al., 1999). In addition, the biochemical properties of muscle fibers differ among species. For example, in large animals, similar muscles contain more slow type MHCs than in small animals. In rat (Kirkeby, 1996), cat (Taylor et al., 1973; Kirkeby, 1996) and rabbit (Bredman et al., 1992), masticatory muscles are predominantly composed of type II fibers, whereas human jaw muscles (Eriksson and Thornell, 1983; Sciote et al., 1994; Korfage and Van Eijden, 1999, 2000) contain a relatively larger proportion of type I fibers. As is the case in the rabbit masseter, MHC-IIB is not found in human masticatory muscles. The absence of MHC-IIB indicates that human masticatory muscles are not very fast. In contrast, a superfast MHC-IIM has been demonstrated in masticatory muscles of non-human primates and carnivores (Rowlerson et al., 1983; Hoh and Hughes, 1991). Because of these differences between human and animal masticatory muscles, care should be taken to extrapolate data on animal contractile properties to human masticatory muscles.

**Fatigability**

For a motor unit, the ability to maintain force during long term contractions is usually expressed by the fatigue index. This index is defined as the ratio between the motor unit force after and before several minutes of exhaustive stimulation. The only data
on fatigability of motor units in human masticatory muscles was reported by Nordstrom and Miles (1990) on the masseter muscle. Using the spike-triggered averaging technique, only two of their thirty seven units were classified as slow and the remaining units as fast. The units showed a wide range of fatigability, and no significant correlation was found between fatigability and twitch amplitude or contractile speed.

By exposing rabbit masseter motor units (n=41, fast type) to a standard stimulation regimen to evoke fatigue, Kwa et al. (1995b) found that, except for one motor unit, all motor units in the rabbit masseter had fatigue indices larger than 0.75 and could thus be classified as fast, fatigue resistant (FR). The mean fatigue index was 1.04 (Table 4) and a relatively large number of motor units had indices greater than 1. Hence, repetitive stimulation does not always lead to a decrease in force. Thus far, the underlying mechanism of this phenomenon, called potentiation, has not been elucidated. Potentiation is highly dependent on the nature of activation (Kernell et al., 1983b). Fast units show greater potentiation than slow units (Parry and DiCori, 1990; Kwa et al., 1995b). Taylor et al. (1973) reported post tetanic potentiation to be present in cat masseter and temporalis muscle strips; after a 10 s period of tetani (1 train/s, train duration 0.5 s, frequency 100 Hz), twitch force increased to 130% of its pre-tetanic value. The same authors tested fatigability by the application of slightly different stimulus trains (1 train/s, train duration 330 ms, frequency 55-90 Hz) and reported a force magnitude decrease after one minute of about 25% of the initial value. These results indicate that the masseter and temporalis muscle in the cat are relatively fast and fatigable.

The ability of motor units to resist fatigue is correlated with the metabolic properties of the constituent muscle fibers. Muscle fibers of fatigue resistant motor units (S and FR) contain a substantial amount of aerobic end-oxidation enzymes. Muscle fibers of fatigable motor units (FF) are rich in glycolytic enzymes and low in enzymes of aerobic oxidative metabolism (for review: see Pette and Vrbová, 1985). Differentiation between various types of fibers can be accomplished by histochemical techniques like detection of succinate dehydrogenase (SDH) activity which is relatively high in S and FR fibers and low in FF fibers. The content of the aforementioned enzymes in masticatory motor unit muscle fibers shows a continuous spectrum. The discrimination between fatigable and fatigue-resistant motor units, therefore, is arbitrary.
Distribution of fiber and motor unit types

Immuno- and enzyme-histochemical studies in both animal and human masticatory muscles point to a heterogeneous distribution of fiber types across the muscles. Generally, the more deep and anterior muscle regions contain relatively large numbers of slow fiber types, whereas the more superficial and posterior regions contain relatively large numbers of fast fiber types (pig: Herring et al., 1979; rat: Rokx et al., 1984; monkey: Clark and Luschei, 1981; rabbit: Bredman et al., 1990; man: Eriksson and Thornell, 1983; Korngel and Van Eijden, 1999, 2000). This heterogeneity was physiologically confirmed in the rabbit masseter by Turkawski et al. (1998), who found that the slowest contracting motor units were located in the anterior portion.

The heterogeneous distribution of motor unit types correlates with the heterogeneous activation patterns of the masticatory muscles during chewing or biting (pig: Herring et al., 1979; rabbit: Weijls and Dantuma, 1981; man: Blanksma and Van Eijden, 1990, 1995; Blanksma et al., 1992, 1997). For example, in general, the anterior regions of the temporalis muscle are more intensively used than its posterior regions and the deep regions of the masseter are more intensively used than its superficial regions. In general, more slow type fibers are found in muscle areas that are more intensively used, indicating an adaptation to functional needs. It should be noted that MHC content of motor units can be altered by exercise (Roy et al., 1991; Adams et al., 1993), chronic electrical stimulation (Pette and Vrbová, 1992), and changes in thyroid and androgen hormone levels (Lyons et al., 1986; Hughes et al., 1993). Hence, transition of a faster fiber type to a slower fiber type, or vice versa, may occur. During such a transition, fibers may contain more than one type of MHC and are referred to as hybrid. Chronic low-frequency stimulation has been demonstrated to induce a fast-to-slow transition (Pette and Vrbová, 1985; Pette, 1990; Rafuse et al., 1997). Thus, fast type fibers can transform into slow type fibers if they are chronically stimulated and this could explain why there are more slow type fibers in areas that are more intensely activated (Hensbergen and Kernell, 1997). Compared to fast type motor units, slow type motor units produce less force and are recruited predominantly in the initial phase of force development. It can therefore be expected that muscle portions with a high proportion of slow type motor units are better equipped to regulate the magnitude of the force produced during chewing or biting than muscle portions with a low proportion of slow units.
Motor unit activation

The magnitude of force produced by a muscle can be modulated by the central nervous system by two mechanisms: (1) varying the number of motor units that are recruited (recruitment gradation) and (2) varying the discharge rate of action potentials of each motor unit (rate gradation). Although both mechanisms occur simultaneously, their relative contributions differ. In general, recruitment gradation is more applicable at low force levels and rate gradation at high force levels (Hennig and Lømo, 1987; Fournier and Sieck, 1988). However, the relative contribution of both mechanisms to the force output at a particular force level is muscle specific (Mori, 1973; Monster and Chan, 1977). The general principle may also be applicable for the human masticatory muscles. At relatively low bite force levels (0-20% of the maximum), between 50% (Goldberg and Derfer, 1977; Hannam and McMillan, 1994) and 87% (Scutter and Türker, 1998) of the masseter motor units are recruited.

Recruitment gradation

The excitability of a motoneuron is inversely related to the size of its perikaryon. This size varies among motoneurons in a motor pool. Small motoneurons reach their activation threshold and start firing earlier than large motoneurons. Therefore, as the excitatory input to a motor pool increases, the motoneurons of this pool are recruited in an orderly sequence, i.e., in order of motoneuron size (Henneman et al., 1965). With a decrease in excitatory input, the motoneurons are de-recruited in the reverse sequence. As the size of the motoneurons is closely related to the size of the motor units, i.e., small motor units are innervated by small motoneurons and large motor units by large motoneurons, the recruitment of motor units is also assumed to occur in the same orderly sequence. At low muscle force, the smaller type S motor units are recruited first and with an increase of force the larger type F motor units are added. As the type S motor units are slower and produce less force and are less susceptible to fatigue than type F motor units, this progressive recruitment allows for a fine control of muscle force and a large resistance to fatigue at low contraction levels.
A parameter which is commonly used to characterize motor unit recruitment as a function of motor unit size is the recruitment threshold (also force or activation threshold), \textit{i.e.}, the muscle force at which the unit is first activated. Generally speaking, because the force produced by the individual masticatory muscles cannot be measured directly, the recruitment threshold is routinely defined by the bite force level at which the unit is activated. The size of the motor units has been estimated by the amplitude of the motor unit action potential (see for example: Yemm, 1977; Goldberg and Derfler, 1977; Desmedt and Godaux, 1979; Scutter and Türker, 1998). Also twitch force, determined by spike-triggered averaging, has been used to estimate the size of the motor units, but this technique is less accurate for this purpose (see above).

Motor unit action potential can be defined as the sum of the action potentials propagated by the muscle fibers that belong to that motor unit (Stålberg et al., 1996). The action potentials of the single fibers differ in amplitude and in frequency content and they are temporally and spatially dispersed. For this reason, motor unit action potentials differ in shape. Differences in amplitude are clearly associated with differences in motor unit size, \textit{i.e.}, differences in dimensions of individual fibers as well as the number of active fibers (Burke and Tsairis, 1973). The size and shape of motor unit action potential also changes with muscle length (human: Miles et al., 1986; rabbit: Turkawski and Van Eijden, 2000c). Also, the distance between active fibers and electrodes plays an important role (Fuglevand et al., 1992). It should be noted that at force levels larger than 30\% of maximum force, it becomes increasingly difficult to distinguish single motor unit action potentials in the interference EMG. Recently, information about the relationship between motor unit action potential parameters and motor unit size and contractile properties not hampered by such interference EMG has become available through stimulation of rabbit masseter motor units (Turkawski and Van Eijden, 2000a,c). Motor units producing larger forces tended to have action potentials with larger amplitudes and faster motor units had action potentials with shorter durations.

In the majority of the above-mentioned studies on motor unit recruitment of masticatory muscles, motor unit size and motor unit threshold are related to bite force magnitude, assuming that the contribution of the relevant muscle to the production of total bite force is linear with the bite force. The masticatory system, however, is mechanically redundant, implying that more than one muscle contributes
to the production of a particular bite force. Therefore, this contribution is not unambiguous and varies with, for example, the direction of bite force (Van Eijden et al., 1990), tooth location (Van Eijden et al., 1993), jaw opening angle (Koolstra et al., 1988), and muscle and sarcomere length (Koolstra and Van Eijden, 1997). Also, the motor unit's recruitment threshold is not fixed but depends on a number of parameters, including contraction velocity (Büdingen and Freund, 1976) and muscle length (Miles et al., 1986). Therefore, one should be careful in using the bite force recruitment threshold as the only criterion for characterizing motor units. These factors can be excluded if muscle force can be estimated more directly, and therefore the surface EMG recruitment threshold determined from the activity of the muscle under consideration has been advocated as an alternative (Scutter and Türker, 1998).

Henneman's principle of an orderly recruitment of single motor units (Henneman et al., 1965) has been reported for various jaw closing muscles, with the smallest units being recruited first and the larger units being progressively recruited as force increases (human masseter and temporalis: Yemm, 1977; monkey temporalis: Clark et al., 1978; monkey masseter, temporalis and medial pterygoid: Lund et al., 1979; human masseter: Goldberg and Derfler, 1977; Desmedt and Godaux, 1979; Scutter and Türker, 1998). The results of these studies show that motor units with high recruitment thresholds tend to produce larger twitch forces than units with lower thresholds. Motor unit action potential amplitude, twitch tension and recruitment threshold have been reported to be mutually proportional (Goldberg and Derfler, 1977; Clark et al., 1978; Scutter and Türker, 1998). In accordance with these observations are the results of Turkawski and Van Eijden (2000a,c), that revealed a correlation between motor unit force and action potential amplitude upon single motor unit stimulation of rabbit masseter motor units.

The recruitment threshold of motor units appears not to be fixed but depends on the motor task. For example, the threshold has been reported to vary with bite force direction (Hattori et al., 1991) and the duration of muscle contraction (Nordstrom and Miles, 1991). Furthermore, an increase in threshold has been associated with an increase in jaw gape and muscle length (Miles et al., 1986) and with a decrease in muscle contraction velocity (Desmedt, 1983). Many mechanisms responsible for this modulation of recruitment threshold have been proposed, including changes in excitability of the motoneuron pool, inhibition mediated by Golgi
tendon organs, presynaptic inhibition, and Renshaw inhibition (for an overview: see Hannam and McMillan, 1994; Scutter and Türker, 1998). In addition, the recruitment order also appears not to be fixed. For example, in case of excitation input to the motoneuron pool, motor units are more or less simultaneously recruited (Desmedt, 1983). A reverse recruitment order has also been reported during slow ramp contractions (Hannam and McMillan, 1994).

**Rate gradation**

When a motoneuron is activated by a series of pulses with a sufficiently high frequency, the successive twitches will summate and fuse, resulting in a larger force than that of the individual twitches. The relationship between stimulation frequency and motor unit force is described by a force-frequency curve (Fig. 4) which has a sigmoid shape (Kernell et al., 1983b; Botterman et al., 1988; Bakels, 1993). In the first low plateau of the curve, stimulation rate is low and no fusion of twitch forces

![Figure 4](Image)

Figure 4.
Force-frequency curve of a slow (left curve) and fast (right curve) motor unit of cat peroneus longus muscle. The steep part of the curve is the optimal part for force modulation by rate gradation. Slow units seem to be more sensitive to rate gradation than fast units. Adapted from Bakels (1993).
occurs. In the beginning of the steep part of the curve, gradual summation of twitches starts. In the human masseter, summation of twitches is believed to start at 7-10 Hz (Nordstrom and Miles, 1989). At the so-called fusion frequency, complete fusion occurs. A further increase of the discharge rate goes along with a further increase in the force until maximum tetanic force is reached at the second plateau. Thus, the force output of a single motor unit can be modulated by the discharge frequency of its motoneuron. The steep part of the curve is the most optimal and most sensitive part for force modulation by rate gradation, as in this portion of the curve a small change in discharge rate results in a considerable change of force. The rate modulation sensitivity is determined by the steepness of the curve.

The force-frequency curves of slow and fast motor units are different. First, in slow units, the frequency at which fusion starts and the fusion frequency are lower than in fast units. In other words, at a particular stimulation frequency, slower motor units display more fusion than that found in faster motor units. In rabbit masseter motor units (n=20), Kwa and Weijs (1999) found a fusion frequency of 37 Hz for the slowest (twitch contraction time: 30 ms) and a fusion frequency of 55 Hz for the fastest motor units (twitch contraction time: 17 ms) examined. These fusion frequencies are lower than those reported for hindlimb muscles, where values of up to 100 Hz have been reported for the fastest units (Celichowski and Grottel, 1995).

Second, the inclination of the curve’s steep portion is larger in slow than in fast units (Kernell et al., 1983b; Kwa and Weijs, 1999). Hence, slower units seem to be more sensitive to rate gradation than fast units and need a smaller change in stimulation frequency to achieve the same relative force change. Finally, the difference between twitch force (first plateau of the curve) and maximum tetanic force (second plateau of the curve) is smaller for slow than for fast units. The relative difference can be expressed by the twitch-tetanus ratio. Kwa and Weijs (1999) found twitch-tetanus ratios in rabbit masseter motor units to range between 0.19 and 0.84 and indeed found that slower units had larger ratios than faster units.

The range of individual motor unit discharge frequencies during natural functioning is relatively small and the reported frequencies are relatively low compared to those studied in experiments in which force-frequency relationships have been determined (Miles, 1994). Kwa and Weijs (1999) observed spontaneous firing rates in the rabbit masseter between 15 and 33 Hz, which is comparable to the rates reported for motor units in the temporalis muscle of the rhesus monkey (Clark
et al., 1978). This would imply that, normally, fusion of twitches does not occur in rabbit masseter motor units and that rate gradation is effective predominantly in slower units. Eriksson et al. (1984) reported firing rates for human and temporalis motor units during voluntary isometric contractions when force increases from slight to moderate. The lowest rate of firing was between 5 and 8 Hz, and the highest rate, between 20 and 25 Hz. Beyond this force, other units often disturbed the recordings and therefore higher force levels could not be investigated.

**Heterogeneous activation**

It has been known for quite sometime, especially in anatomically complex muscles with large attachment areas, that different muscle regions can be activated more or less independently from each other, depending on the task. In some muscles, the independent activation of discrete muscle regions has been associated with different innervation patterns. This feature has been termed compartmentalization (English and Letbetter, 1981). For various aspects of the functioning of complex muscles and of compartmentalization, the reader is referred to the literature (English and Weeks, 1984; English and Weeks, 1987; Stuart et al., 1988; Windhorst et al., 1989; Chanaud and Macpherson, 1991; Chanaud et al., 1991a,b; Pratt and Loeb, 1991; Pratt et al., 1991; Sokoloff et al., 1998). In addition, it has been shown that motor units within a given muscle region show task-dependent behavior (Desmedt and Godaux, 1981; Ter Haar Romeny et al., 1984; Hoffer et al., 1987). Hence, the motoneurons in a motoneuron pool of a particular muscle are not likely to be subjected to the same excitatory inputs. Such a heterogeneous activation of the population of motor units in a muscle is not restricted to structurally complex muscles (Milner-Brown et al., 1973; Van Zuylen et al., 1988; Hoffer et al., 1987).

A large number of studies have shown regional differences in muscle activation in both human and animal masticatory muscles and indicate that the activity of various muscle regions is task specific (Weijs and Dantuma, 1981; Herring et al., 1979; Belser and Hannam, 1986; Blanksma and Van Eijden, 1990, 1995; Blanksma et al., 1992, 1997; Van Eijden et al., 1990, 1993; Murray et al., 1999; Weijs et al., 1999). For example, the relative contribution of various muscle regions to the production of bite force changes with the magnitude and direction of the exerted bite force (Van Eijden et al., 1990; Blanksma and Van Eijden, 1990;
Blanksma et al., 1992). In agreement with these observations, masticatory muscle motor units have also demonstrated task-related behavior. Separate groups of motor units are recruited as the task changes (temporalis: Eriksson et al., 1984; McMillan, 1993; masseter: Eriksson et al., 1984; McMillan and Hannam, 1992; lateral pterygoid: McMillan and Hannam, 1989). For instance, most motor units in the posterior superficial part of the masseter muscle, but not in the anterior, inferior, or superficial regions, contribute to tasks involving tooth contact (McMillan and Hannam, 1992). This task specific behavior points to task-related changes in neural drive to motor units and reflects central and peripheral differential influences on a muscle's motoneuron pool. For example, the number of muscle spindles is relatively large in muscle regions containing relatively large numbers of slow type fibers (Rowlerson et al., 1988; Bredman et al., 1991b; Sciotte and Rowlerson, 1998) and this might facilitate differential control of different muscle regions. In addition, the differential regional muscle activation is in accordance with the reported somatotopic distribution of motoneurons in the nucleus of the trigeminal nerve. For example, within the masseter motoneuron pool, the motoneurons for the superficial and deep masseter occupy different regions and motoneurons for the anterior and posterior deep masseter could also be distinguished (rabbit: Matsuda et al., 1978; Weijjs, 1996; guinea pig: Uemura-Sumi et al., 1982; rat: Rockx et al., 1985). The amount of overlap between the regions reported in the various studies varied from none (Matsuda et al., 1978; Uomura-Sumi et al., 1982) to about 50% (Weijjs, 1996). This organization permits a differentiation of descending or afferent input to separate regions in the motoneuron pool. It also enables differential activation between the various muscle portions during the execution of various motor tasks. Within the population of motor units belonging to a muscle region, the recruitment order might be fixed in agreement with the size principle (Riek and Bawa, 1992).

**Mechanical heterogeneity**

The possible selective activation of muscle regions gives a muscle the potential to have numerous lines of action that differ in orientation and position, especially in case of architecturally complex muscles with broad attachment areas, like the masseter, temporalis and pterygoids. Muscles with large attachment areas are mechanically interesting because they have the capability to affect various and
variable degrees of freedom, during selective activation of different muscle parts (Van der Helm and Veenbaas, 1991). Because the masticatory muscles have a complex architectural design with broad attachment areas and with fibers usually not running parallel to each other, they can be approximated by a large number of three-dimensional force vectors, having no common origin or insertion. Therefore, these muscles are able to apply forces to the lower jaw with respect to the upper jaw with six degrees of freedom. Such a mechanical diversity has been shown not only for relatively large muscle portions (Van Eijden et al., 1995, 1996, 1997), but also for single motor units (Turkawski et al., 1998; 2000b). Representing such a complex muscle by a single force vector would be appropriate in the case of homogeneous activation. However, it underestimates its potential mechanical effect in case of selective activation of specific muscle regions and motor units.

As motor units in architecturally complex muscles have different intramuscular positions, their length changes and their capability for force production will differ. During, for example, jaw open-close movements motor units situated anteriorly in the masseter are further away from the axis of rotation than posteriorly situated units and, therefore, their sarcomeres will undergo greater excursions. Consequently, changes of motor unit force as a function of jaw angle can be expected to be larger in anteriorly located motor units. Indeed, clear antero-posterior differences in the shape of angle-force curves of masseter motor units have been demonstrated (Turkawski and Van Eijden, 2000b). Compared to posteriorly located motor units, anteriorly located units produced less relative force at occlusion, showed a steeper increase of force with jaw angle and produced larger force at maximum (21°) jaw opening. One of the implications of this heterogeneity is that motor units and muscle portions differ in their capability to participate in particular motor tasks.

Concluding remarks

Morphological and physiological properties of masticatory muscle motor units have been studied in various animals and in human. It must be emphasized that most
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Studies have been confined to the masseter muscle, a jaw-closing muscle. Hence, care should be taken to extrapolate findings since the architectural and physiological properties of the jaw-closing muscles may differ from those of, for example, the jaw-opening muscles. Some of the methods employed have a number of limitations. For example, the spike-triggered averaging technique has been used to study human motor unit twitch forces. The twitch profiles obtained with this technique may be biased by a number of factors, including co-activation of muscles. The method of extracellular stimulation, applied in the rabbit masseter, may be biased due to a preference for fast motor unit excitation. Furthermore, care should be taken to extrapolate data on contractile properties of animal masticatory muscles to human masticatory muscles, because of differences in biochemical composition of muscle fibers.

Masticatory muscle motor units differ considerably from limb and trunk muscle motor units. Thus far, it is not known why the masticatory muscles have so many different properties and the mechanisms that are responsible for these differences have still not been elucidated. In masticatory muscles, the organization of motor control is more localized and the classification of motor unit types is less distinct. These features imply that, in masticatory muscles, a finer gradation of force and movement is possible than in limb and trunk muscles. Differences among motor units with respect to contraction force and contraction speed seem to be primarily related to the variation of physiological cross-sectional area and to the variation of MHC isoform content, respectively. An intriguing feature is that the biochemical properties of muscle fibers, including the MHC isoform content vary with, for example, age, gender, activation intensity and hormone level, as has been demonstrated for limb muscles. Thus far, however, there is a lack of information whether such variation is applicable to masticatory muscle motor units and to what extent. Such information is important for two reasons. First, it can shed light on the mechanisms that determine the physiological properties of masticatory motor units and on the factors that are responsible for the heterogeneous distribution of motor unit types across muscles. Second, it is not unrealistic to expect that adaptational changes of, for example, MHC content could occur in clinical situations, such as bruxism, aberrant dental occlusion, orthodontic and gnathosurgical interventions, abnormal craniofacial morphology, and age-related or edentulous masticatory muscle and jaw bone atrophy. Such changes could change motor unit physiological
properties, affecting the loading of the jaws, teeth and temporomandibular joints. The capacity for adaptational changes may differ across motor units, muscles and animals.