Motor control of the upper trapezius

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Preface

"Muscles" he asked.
"Sure I know muscles" I replied.

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- Friends, colleagues and staff on campus

Extra special thanks go to my dear Sigrun and my daughter Åsne for support, cheers and extra motivation.

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Westad C, Westgaard RH & De Luca CJ (2003). Motor unit recruitment and derecruitment induced by brief increase in contraction amplitude of the human trapezius muscle. *J Physiol* **552**, 645-656

Paper II:
Westad C, Mork PJ & Westgaard RH (2004). Firing patterns of low-threshold motor units in feedback-controlled contractions and vocational motor activities. *Exp Brain Res* **158**, 465-473

Paper III:
Westad C & Westgaard RH (2005). The influence of contraction amplitude and firing history on spike-triggered averaged trapezius motor unit potentials. *J Physiol* **562**, 965-975.

Paper IV:
Westgaard RH, Bonato P & Westad C. Respiratory and stress-induced activation of low-threshold motor units in the human trapezius muscle. *Manuscript*

Abstract

Background

The main objective of this thesis was to establish new insight into the motor control of low-threshold motor units in the trapezius muscle. Special attention was given to motor unit recruitment threshold related to firing behavior.

The extensive literature on motor control mainly concerns firing behavior of extremity muscles. Motor control of the upper trapezius shows features that indicate deviations from the control scheme generally assumed to apply to muscles of the extremities. Knowledge of motor control of the trapezius is important in a pain development perspective, since shoulder/neck complaints are frequently localized to this muscle.

Methods

Constant amplitude contractions of 2 to 30 min duration, with amplitudes between ~2–7% of maximal voluntary contraction (2-7% EMG_{max}), were used to study time-dependent changes in motor unit firing. Transient force increases reaching 15-20% EMG_{max} were superimposed on some of these contractions in an attempt to induce motor unit substitution. Sinusoidal contraction profiles were used to study firings in response to dynamic contractions. Motor unit firing was studied in 10 min contractions with vocational (typing) tasks and mental stress. The spike-triggered averaged (STA) technique was used to examine motor unit potentials and their dependence on contraction amplitude and firing history.

The individual motor unit firings were recorded by intramuscular fine wire electrodes, while simultaneously recording the surface electromyographic (SEMG) signal. The Precision Decomposition technique was used to identify individual motor units with near 100% accuracy.

Results

The results show that some low-threshold motor units stopped firing at the end of the EMG-pulses while motor units with initially higher threshold were recruited or stayed active. The lowest threshold motor units showed only brief silent periods.

The mean firing rate increased from 10.5 to 12.5 pulses per second (pps) in response to contraction amplitudes of <2% and >4% EMG_{max}, but the mean firing rate was the same for all motor units regardless of task and recruitment threshold given the same SEMG amplitude. There was a strong rate modulation in dynamic contractions.

STA-derived motor unit potentials indicated that motor units recruited below 10% EMG_{max} had similar area at the same contraction amplitude. However, the area increased four-fold when SEMG amplitude increased from 1.5 to 10% EMG_{max}.

Motor unit synchronization showed an average of 2.8% additional firings within ±2 ms of the triggering motor unit, estimated by peristimulus time histograms (PSTHs).

A surprising finding was respiratory modulation of the firing rate at low contraction amplitudes. This modulation was attenuated by induced mental stress.

Conclusions

Transient force increases promote derecruitment of motor units and may be attributed to inactivation of non-inactivating inward currents (plateau potentials). Silencing of motor units can be considered a protective mechanism to reduce the metabolic load on low-threshold motor units.

The similar firing rates in sustained contractions independent of task and recruitment threshold suggest that the duration and pattern of silent periods are the most important variables to investigate in relation to motor unit over-exertion and subsequent pain development.

The strong rate modulation in response to dynamic contractions indicates a control strategy resembling that of extremity muscles.

STA-derived motor unit potentials indicate that units recruited below 10% EMG_{max} are of similar size, thus suggesting a deviation from the Henneman size principle. This may be an adaptation to postural functionality. The increase in STA-derived potentials is largely due to motor unit synchronization. This points out limitations for this method when quantifying motor unit size, numbers and conduction velocity.

Finally, respiratory modulation of firing rate and the attenuation by mental stress suggest at least two different sources of autonomic input that may facilitate motor unit activity.

Background

Reasons to study trapezius motor control

The vast literature on motor unit firing behavior in human muscles is mainly derived from short duration experiments on extremity muscles. Motor unit firing patterns in long-duration experiments, particularly experiments mimicking natural use of postural muscles, have not been extensively studied.

Some interesting observations of low-threshold motor units in the upper trapezius muscle indicate motor control properties that deviate from the hierarchical organization of firing pattern generally assumed to apply during muscle contractions of extremity muscles. In extremity muscles increasing contraction amplitude activates progressively larger motor units (Denny-Brown & Pennybacker, 1938 ; Henneman, 1957), and motor unit firing rates traces the variations in contraction amplitude (Westgaard & De Luca, 2001). The deviations of the trapezius include frequent changes to motor unit recruitment order, periods of motor unit inactivity and differences in firing pattern in slow ramp contractions compared to extremity muscles (Westgaard & De Luca, 1999; Westgaard & De Luca, 2001). As a muscle serving postural demands, the trapezius is required to sustain long periods of motor unit activity (Jensen *et al.*, 1993a). With a hierarchical recruitment order, low-threshold motor units in the trapezius are subject to high metabolic loads because the same population of motor units, according to the Henneman size principle, is always activated first. It has been proposed that over-exertion of low-threshold motor units has a detrimental effect on the motor units, eventually leading to pain development (Hägg, 1991). This has been supported by the observations of ragged red fibers and moth-eaten fibers in type I muscle fibers (Larsson *et al.*, 2000). These phenomena are signs of disturbed metabolic functions and are thought to result from excessive activation of muscle fibers. Moreover, the trapezius is shown to be more responsive to mental stress, in terms of elevated surface EMG, compared to most other muscles (Wærsted & Westgaard, 1996), with long-duration trains of motor unit activity observed at low activity levels in experimental conditions of attention demanding tasks (Wærsted *et al.*, 1996).

Considering these aspects, the trapezius may require different control strategies to that of the generally more short-duration, phasic use of extremity muscles.

Trapezius anatomy

The human trapezius muscle has a complex anatomy with multi-pinnate fiber orientation. It is the most superficial of the upper back muscles and derives its name from the irregular, four-sided shape that its fibers occupy (Fig. 1A). The trapezius is a large, flat muscle which origin runs from the superior nuchal line, then passing to the ligamentum nuchae down to the level of C7, the spinous processes of vertebrae C7 to T12 and the supraspinous ligaments. Between C6 and T3, the origin is aponeurotic and covers a diamond-shaped interval which extends outwards to the edge of T1.

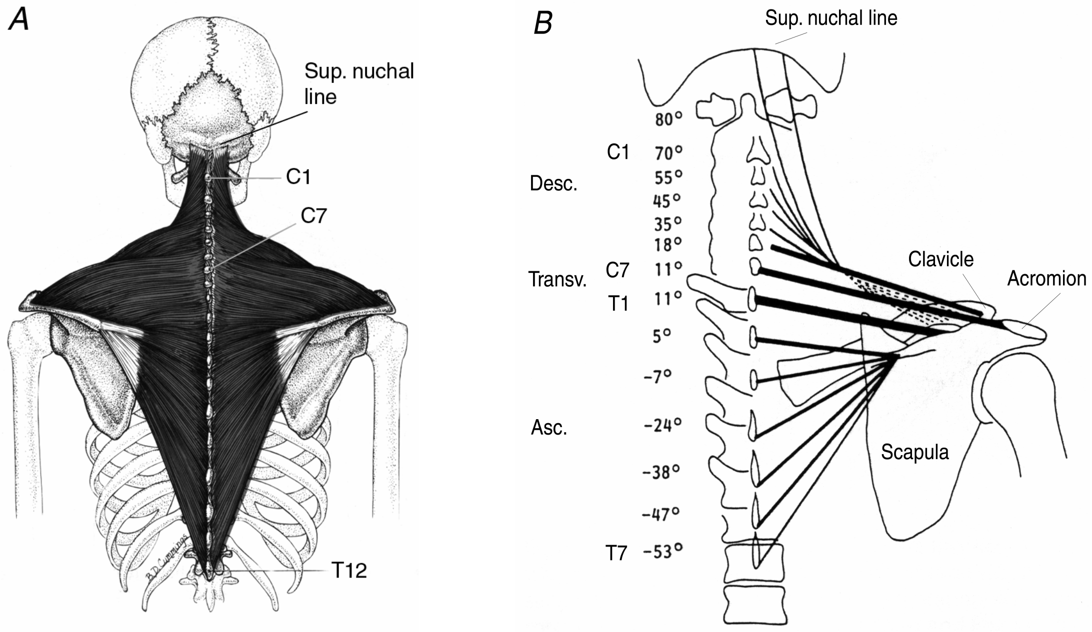

Figure 1. Trapezius muscle (*A*) and muscle fiber orientation (*B*). (*A* modified from Simons *et al.*, 1999, *B* modified from Johnson *et al.*, 1994)

Trapezius converges on the pectoral girdle and may be divided into three groups of fibers of different origin (Fig. 1B):1) Descending fibers: pass inferolaterally from the skull and cervical spine to insert systematically on the posterior border of the lateral third of the clavicle such that the fascicle from the superior nuchal line assumes the most anterior position, then fascicles from the upper and lower half of ligamentum nuchae, while fibers from the C6 spinous process insert on the distal corner of the clavicle. 2) transverse fibers: pass horizontally from the level of C7 to T1 to insert on the inner border of the acromion and the superior lip of the crest of the scapular spine.

3) ascending fibers: via a tendon into the tubercle on the medial end of the spine of the scapula (Johnson *et al.*, 1994; Standring, 2005).

Biomechanical actions of the trapezius

The trapezius is involved in a large range of movements, most of them in synergy with other muscles. Extension of the neck is produced by bilateral contraction of the descending trapezius fibers, while lateral flexion of the neck is produced by unilateral contraction of the descending fibers. The descending trapezius fibers act to draw the clavicle, acromion and spine of the scapula backwards and medially and have only a limited capacity for upward movement of the clavicle. The elevation of the scapula is mainly produced by contraction of transverse fibers of the trapezius but after the movement has been initiated by other muscles, notably the serratus anterior. This motion not only permits shrugging of the shoulders, but also prevents depression of the shoulders when the pectoral girdle is being pulled downwards e.g. when carrying heavy weights in the hands. Furthermore, lateral rotation of the scapula permits rotation of the glenoid cavity superiorly around a point near the base of the scapular spine. This movement is important in abduction at the glenohumeral joint. The role of the ascending trapezius fibers is to move the base of the spine inferiorly and to maintain horizontal and vertical equilibrium of the scapula (Johnson *et al.*, 1994). The nerve supply to the trapezius derives mainly from the spinal accessory nerve but also from the deep muscular branches of the cervical plexus (Weisberger, 1987; Kierner, 2000).

Recording point structure

The upper trapezius, which has been studied in this thesis, has a sheet-like appearance with mean thickness of 5 mm at the preferred electrode position (Jensen *et al.*, 1994). The C7 fascicle of trapezius has 49 - 76% type I fibers (Lindman *et al.*, 1990; Lindman *et al.*, 1991). The physiological cross-section of the C7 fascicle is 2.2 cm^2 (Johnson *et al.*, 1994), corresponding to more than 50 000 muscle fibers if mean fiber size is 4000 μm^2 (Larsson *et al.*, 2001). Here it must be emphasized that the number of fibers is a crude estimate as there are many uncertainties in this type of calculation (Enoka, 1995; Enoka & Fuglevand, 2001). The number of trapezius motor units and motor unit innervation numbers are not known. Assuming a similar range and distribution of innervation numbers of type I and type II motor units as derived from FDI, more than 90% of the motor unit pool innervates type I fibers in the trapezius (Enoka & Fuglevand, 2001).

Motor control principles

Muscle force development

The force exerted by a muscle is dependent on the number of recruited motor units and their discharge rate. There is large variation between muscles in terms of the number of motor units, motor unit size, innervation ratio, spatial distribution of muscle fibers, cross-sectional area of the fibers, fiber length and fiber type composition. For instance, small muscles tend to have lower innervation ratios (i.e. fewer muscle fibers innervated by the same motoneuron) than large muscles and this ratio generally relates to the ability of the muscle to finely grade muscle force (Enoka, 1995). The maximal force generated by any given motor unit depends on the number of muscle fibers innervated by its motoneuron and the maximal force the innervated muscle fibers can generate. The cross-sectional area of a muscle fiber and the force capacity per unit of cross-sectional area determines the maximal muscle fiber force (Enoka, 1995).

Control of muscle force output

Perhaps the best known principle in motor control is the fixed recruitment order of motor units in low-force and isometric contractions, progressively recruited from small to large motor units (Denny-Brown & Pennybacker, 1938). The proposed mechanism behind the orderly recruitment scheme is the difference in surface area of the soma and dendrites of the motoneuron, which co-varies with motor unit size (Henneman, 1957), known as the “Size Principle”.

Although the size principle is generally accepted to be valid for a variety of muscles, deviations from the orderly recruitment pattern can occur (Nordstrom & Miles, 1991). A number of studies have reported substitution of motor units (Kato *et al.*, 1981; Sjøgaard *et al.*, 1986; Fallentin *et al.*, 1993; Westgaard & De Luca, 1999). The concept of substitution is the recruitment of higher threshold motor units to replace lower-threshold units that have stopped firing. The mechanisms for reversal of recruitment threshold between motor units are not yet fully understood. Functionally it has been suggested that motor unit substitution protects motor units from excessive fatigue during low-amplitude sustained contractions (Person, 1974).

A possible physiological mechanism to explain increase and lowering of the recruitment threshold of low-threshold motoneurones is non-inactivating inward currents (plateau potentials) (Hounsgaard *et al.*, 1988). Plateau potentials are suggested due to voltage dependent non-inactivating Ca^{2+} conductance, reduced K^+ conductance (Hounsgaard & Kiehn, 1985), or by procedures that shift the equilibrium potential for K^+ in the depolarizing direction (Schwindt & Crill, 1980), resulting in a stable membrane potential that is more depolarized than the resting membrane potential (Kiehn & Eken, 1998). A prominent indication of plateau potentials in motoneurones is the abrupt increase in firing rate that is lowered to a stable firing rate within a few seconds and the continuing activity even at lower central drives compared to that of its recruitment (Eken & Kiehn, 1989; Kiehn & Eken, 1998; Hornby *et al.*, 2002). This self-sustained activity pattern can be initiated by a transient depolarization of motoneurones ('on' stimulus) and terminates either spontaneously after a few minutes or by brief inhibition ('off' stimulus) (Kiehn & Eken, 1998). Slow motoneurones tend to have the onset of plateau potentials below or at the threshold for action potentials (Lee & Heckman, 1998). This may allow plateau-initiated low-threshold motor units in stable contractions to maintain firing by self-sustained activity while motor units with a longer continuous activation history and lower recruitment threshold are silenced. The inhibitory actions of, e.g. Renshaw cells could potentially act as an off stimulus resulting in silencing of motor units. In this scheme motor axon collaterals activate Renshaw cells, which then respond with an inhibitory action on the motor neuron itself leading to a reduction in the net excitatory input to the motor unit. Each Renshaw cell receives excitatory input from many homonymous and synergistic motoneurones (Katz & Pierrot-Deseilligny, 1998). Both excitatory and inhibitory activity is received from segmental pathways and descending tracts. The Renshaw cells project onto α-motoneurones and γ-motoneurones as well as onto Ia inhibitory interneurones and other Renshaw cells (Renshaw, 1941; Ellaway, 1971; Hultborn *et al.*, 1971). It is the net result of activation in this complex system that determines the strength of the Renshaw cell inhibition onto the motoneuron.

A motoneuron action potential normally activates all muscle fibers it innervates. Therefore, the force output of the particular motor unit depends on the summed force generated by the innervated muscle fibers. Whether the motor unit is activated or not depends on its recruitment threshold relative to contraction amplitude. The recruitment of a motor unit is determined by intrinsic properties of the motoneuron and the net sum of all excitatory and inhibitory input of both central and peripheral origin to that neuron. In this way, a command signal from the CNS to the motoneuron pool may activate some motor units while others remain silent. The same signal may also result in different firing rates between motoneurones in the same pool determined by different susceptibility to excitation (De Luca & Erim, 1994). Under normal circumstances the firing rate of motor units is between 8 and 30 pulses per second (pps). At the lowest firing rates each motoneuron action potential results in a single twitch contraction of the innervated muscle fibers. At higher firing rates there is a temporal summation of twitches producing greater force than that of a single twitch. At still higher firing rates the muscles enters a state of unfused tetanus before eventually reaching totally fused tetanus where single twitches are no longer apparent. Motoneuron firing rates above that which gives totally fused tetanus (~30-40 pps) has no additional force generating effect (Freund, 1983). The maximum maintained firing rate in slow-ramp contraction of limb muscles is between 15 and 35 pps, which is the firing rate necessary to use the full contractile capacity of the muscle fiber (Freund, 1983). Under certain conditions, e.g., when rapid contractions are required or in some ballistic movements, short duration bursts of firing rates as high as 120 pps has been observed (Tanji & Kato, 1973; Desmedt & Godaux, 1978). Doublets (50 pps or higher firing rates) have been observed in extensor digitorum communis (EDC) during finger tasks and computer mouse work (Søgaard *et al.*, 2001). Presumably this is to rapidly exert the required force in a given task, although it has been suggested that, at least in cat intercostal muscles, initial doublets is not always functionally important (Kirkwood & Munson, 1996).

Provided the firing of individual motor units is not synchronized the movement of the muscle is smooth even a low force levels. As the degree of synchronization increases, as is the case in some pathological conditions (Datta *et al.*, 1991) or during fatiguing contractions (Kleine *et al.*, 2001), the movements become increasingly jerky. Some degree of synchronization is always present dependent on task performed (Sears

& Stagg, 1976; Bremner *et al.*, 1991a). For instance, synchronization was found to be greatest during finger extension and on motor units acting on adjacent fingers; least during finger flexion and on muscles acting on widely separated fingers. Furthermore, motor units acting on medial fingers were more synchronized than units acting on lateral fingers (Bremner *et al.*, 1991b). A likely source of synchrony between motor units in distal extremity muscles is shared input to motoneurones from branched axons (Kirkwood & Sears, 1974; Sears & Stagg, 1976; Bremner *et al.*, 1991b). The most important possibly from widely divergent monosynaptic projections from motor cortical cells (Farmer *et al.*, 1993).

Pain development

The most common location for chronic muscle pain is the shoulder and neck region (Andersson *et al.*, 1993; Simons *et al.*, 1999; Ashina *et al.*, 2003). The physiological mechanisms causing pain have been the target of many investigations and the factors proposed to be responsible for muscle pain development are numerous. Reduced level of ATP and ADP (Larsson *et al.*, 1988) as well as insufficient peripheral circulation in the trapezius has been identified as potentially important factors for pain development (Larsson *et al.*, 1990; Larsson *et al.*, 1994). One of the most popular hypothesis to link the development of pain in the trapezius to motor activity is the Cinderella hypothesis (Hägg, 1991). This hypothesis is based on the Henneman size principle. It implies that the same low-threshold motor units are always recruited first during a contraction and keep firing until total muscle relaxation, resulting in excessive strain on these motor units. Thus, the metabolic load on individual motor units could be detrimental to the innervated muscle fibers, resulting in excessive fatigue, structural damage and subsequent pain development. The finding of ragged red fibers and moth-eaten fibers in type I muscle fibers is argued to indirectly support this hypothesis (Larsson *et al.*, 2000). These fibers show mitochondrial dysfunctions thought to result from prolonged activation of a selected population of motor units.

The individual projects of the present thesis were not designed to determine the cause of trapezius muscle pain. However, if over-exertion of low-threshold trapezius motor units is a cause for shoulder pain, it is important to understand the motor control scheme that is applied to the trapezius, in particular that which promotes silent periods and substitution of motor units. Furthermore, assuming the substitution phenomenon is indeed important for the development of muscle pain, knowledge about the motor control scheme may be utilized e.g. in an ergonomic perspective to reduce the prevalence or even prevent the development of muscle pain in the shoulder and neck region.

Mental stress responses

Psychological stress has been identified as a risk factor for musculoskeletal disorders in many epidemiological studies (Theorell *et al.*, 1991). Psychosocial problems at work as a risk factor for complaints are more common in the shoulder and neck region than for other body regions (Westgaard *et al.*, 1993). The trapezius muscle exhibits low-level spontaneous muscle activity that tends to increase in experimental situations with induced mental stress (Wærsted & Westgaard, 1996). Trapezius is one of the most responsive muscles to mental stress in terms of an elevated electromyographic (EMG) activity (Wærsted & Westgaard, 1996). Laboratory studies with induced mental stress have shown that low-threshold motor units can be active even in the absence of physical demands (Lundberg *et al.*, 2002). Furthermore, the motor response to stress is dose-dependent so that a higher stress level is associated with higher muscle activity (Wærsted *et al.*, 1994). The muscle activity observed in surface EMG (SEMG) recordings with induced mental stress has shown low amplitude which has been attributed to sustained motor unit activity (Wærsted *et al.*, 1996). Sustained motor unit firing is required for the over-exertion of single motor units according to the Cinderella hypothesis. Thus mental stress may be linked to the development of pain through prolonged invariant motor unit firing patterns.

Objectives

The main objective of this thesis is to establish new insight into the control of low-threshold motor units of the upper trapezius muscle.

The project focused on the following operative aims:

1. Document methods that induce time-dependent changes in recruitment and derecruitment of low-threshold motor units.
2. Determine contraction profiles that induce substitution of motor units.
3. Characterize motor unit firing patterns in dynamic contractions.
4. Describe motor unit firing patterns in response to induced mental stress.
5. Suggest possible sources of excitatory input to trapezius motoneurones.

Methods and materials

Study samples

A total of 24 healthy subjects, 10 males and 14 females volunteered for these studies. The age ranged from 20 to 56 years. The experiments were carried out according to the Declaration of Helsinki and each subject read and signed an informed consent form approved by the local Institutional Review Board.

Intramuscular EMG-signal recording and analysis

In all studies the Precision Decomposition technique (LeFever & De Luca, 1982; De Luca & Adam, 1999) was used to identify motor units. This technique uses template matching, template updating, firing probabilities and superposition resolution to identify the individual firing times of motor units with up to 100% accuracy (Mambrito & De Luca, 1984). Three representations of the motor unit potentials are obtained by the use of a specialized quadrifilar wire electrode. The electrode was constructed by placing four 50-µm nylon coated nickel-chrome alloy wires (‘Stablohm 800A’, California Fine Wire Co, Grover Beach, CA, USA) in a 27-gauge needle. The wire bundle was cut transversely, exposing only the cross-section of the wires. A hook was formed approximately 1 mm from the exposed end of the wire. The needle was inserted to a depth of approximately 10 mm at a location approximately 10 mm medial to the midpoint of a line between the C7 spinous process and the acromion. The needle was removed and the wire bundle remained lodged in the muscle. Three pairs of wires were chosen as the differential input to the amplifiers.

The analysis relies on establishing templates of the three representations of each motor unit. The electrical potentials of each motor unit summarize linearly to establish the interference EMG picked up by the electrodes. The signals were band-pass filtered from 1 to 10 kHz and all EMG signals were stored on a digital recorder (DATaRec-A160, Racal-Heim Systems GmbH, Bergisch Gladbach, Germany). The signals were subsequently reconverted to an analog form and digitized at a sampling rate of 50 kHz on a PC. The Precision Decomposition analysis performs an automated, template-matching process, whereby the templates of individual units are summed with the appropriate time delay to generate a waveform that in the best possible way matches the recorded signal. The result, in terms of firing rate statistics and the decomposition of individual examples of complex waves, is then inspected visually and by several utility programs to ensure that the final results are valid. The automatic mode is much faster but less accurate than the operator assisted mode and in most cases operator interaction is required for 100% accuracy.

The Precision Decomposition analysis is, to our knowledge, superior to any other system for analysis of trains of motor unit potentials existing at the time of experimentation. Most other systems rely on a single recording of action potentials, which are then differentiated on the basis of a height-window discriminator after filtering. Commercially available systems will provide acceptable data over short recording periods and/or very low force levels, but cannot follow individual units in a complex recorded waveform over time.

A typical 30 minute recording consists of up to 100 000 individually recognized motor unit firings. The task of identifying individual motor units in this amount of data is too time-consuming unless the data is of good quality and most of the decomposition is done automatically. This necessitates a controlled experimental environment. Field studies are difficult due to movement-sensitive intramuscular recording electrodes and the need to accommodate rather bulky recording equipment. Therefore all experiments were carried out in a laboratory, and in cases of vocational task procedures an office workplace was built to fit with the equipment while still allowing for natural movement and postures.

SEMG and force estimates

The SEMG signal was detected by using an active differential electrode with two circular recording surfaces (6 mm in diameter, 20 mm inter-electrode distance). The electrode was positioned with the medial recording area 20 mm lateral to the midpoint of the line between the C7 spinous process and the acromion (Jensen *et al.*, 1993b). The SEMG signal was band-pass filtered at 10-1000 Hz. The root-mean-square (RMS) detected SEMG signal was averaged at a time resolution of 0.2 s.

Force developed by the trapezius cannot be reliably detected due to the complex interaction of the muscles controlling shoulder movement. The muscle force output was therefore estimated by use of the RMS value of the detected trapezius SEMG signal. The SEMG signal was calibrated as a percentage of the RMS-detected EMG activity at maximal voluntary contraction (% EMG_{max}). The force was controlled through visual feedback on a screen placed directly in front of the subject. A force profile appeared on the screen, and the subjects traced the profile with their own overlapping RMS-detected SEMG signal.

Recruitment threshold

Initial motor unit recruitment thresholds were determined as the SEMG amplitude at the onset of a train of motor unit firings during ramp contractions. All constant amplitude contractions also started with a slow ramp of approximately 1% EMG_{max} /s inclination before leveling out at the predetermined level. Some motor units were recruited during voluntary brief increases of the force (EMG pulses). When applicable, recruitment threshold was further checked for consistency by comparing initial threshold with threshold determined in ramp contractions following a brief period of reduced SEMG amplitude at the end of the constant amplitude contractions. Recruitment threshold of the motor units were determined separately for each procedure.

Experimental protocols

Ramp force profiles were presented to the subject to investigate motor unit firing behavior in augmenting contractions. The ramp contraction ranged from 10% EMG_{max} within 3 min (0.055% EMG_{max}/s) to 100% EMG_{max} within 25 s (4% EMG_{max}/s). Sinusoidal profiles were used to explore motor unit firing behavior in contractions with anticipated rate modulation. The sine-wave contractions were of 1 min duration with a 6 s period (frequency 0.17 Hz) and with SEMG amplitude varying between ~2 and ~6% EMG_{max}. Dynamic contractions were performed using staircase profiles. The staircase procedure consisted of contractions increasing from 1 to 10% EMG_{max} in steps of 2 or 5% EMG_{max}. Between each step the contraction was maintained at constant amplitude for 1 or 2 minutes. In long-duration constant amplitude contractions with brief increases in the force every minute, a continuously updating bar-plot was used for feedback instead of a set force profile. The duration of these experiments lasted from 5 to 30 min. The contraction amplitude was held around 5% EMG_{max}. Bar-plot feedback was also used for low-force constant amplitude contractions of 2 min duration. Here, the amplitude was chosen so that only a few motor units were detected, typically at ~2% EMG_{max}. The subjects were allowed at least 2 min rest between each procedure.

Vocational tasks

Vocational tasks consisted of three different typing conditions; standard typing, typing with stress and typing with (an ergonomically) poor position. All trials lasted 10 min. The subject was placed in front of a personal computer (PC) and asked to type a text, placed next to the monitor, using a standard keyboard. Chair height, back inclination and table height was individually adjusted to allow an optimal ergonomic posture for the subjects, with arms resting on the tabletop with a 90° angle maintained at the elbow. This posture was used in the first trial with normal (“standard”) typing and in the second typing condition with pressure on performance. In the second typing condition a monetary reward was offered to perform 10% faster than the standard typing task while maintaining the same or improved error rate. In the third typing condition the table top was elevated 10 cm and the keyboard positioned at the edge of the tabletop, eliminating the possibility of resting the arms on the tabletop. Subjects were not given feedback on contraction amplitude in experiments dealing with vocational tasks. Rest periods of at least 2 min were allowed between each trial.

Mental stress test

The mental stress test consisted of a complex, attention-demanding two-choice reaction test presented on the computer screen. An open (“frame”) and a solid (“brick”) quadrangle were placed in a square pattern, and an alphanumeric suggestion on how to move the brick to superimpose on the frame was given (Westgaard & Bjørklund, 1987). The subject responded by pressing one of two keys, “correct” or “wrong”, by the right or left index finger. A new position of the “brick” and “frame” in the square pattern and a new suggestion then appeared. The execution of the test was self-paced, but the subject first carried out the test for 2 min at a steady pace while attempting to maintain a low failure rate. On the basis of this performance the subject was offered a small monetary reward to perform 10% faster with the same or lower failure rate for 10 min. Feedback provided on the computer screen informed the subjects about the response speed (very slow, slow, OK, fast, or very fast) and whether the answer was correct (Wærsted *et al.*, 1994).

Heart rate and respiration frequency

Heart rate and respiration frequency were recorded to indicate the level of mental stress in the reaction test and the typing experiments with induced stress. These recordings were also included in the non-stress protocols for comparison purposes.

An elevated heart rate and respiratory frequency in the induced stress procedures compared to procedures without stress would be regarded as evidence for successfully induced stress.

Summary of results

Heart rate and respiration recordings

Heart rate and respiratory frequency reflected the low level of physical strain in these experiments. The mean values of heart rate and respiratory frequency in the different procedures of 10-min duration ranged from 60 to 75 beats per min (BPM) and 0.3 to 0.35 Hz, respectively (Fig. 2B and C). The procedures with monetary rewards to generate pressure on performance succeeded in establishing the intended condition of moderate stress during the mental stress test (relative to the constant-force procedure) and in typing with stress (relative to standard typing and typing with poor posture). The heart rate was about 5 BPM elevated in the two stress conditions (mental stress vs. constant-force contractions, $p=0.06$; typing with stress vs. the two typing procedures without stress, $p<0.01$). Likewise, mean respiratory frequency was elevated in the conditions with stress relative to reference conditions; however, the respiratory response was more variable and was not significantly different to the corresponding reference conditions. The elevated heart rate and respiratory frequency were maintained throughout the two tasks with induced stress. Respiratory frequency was also elevated in the first min of the standard typing task, potentially indicating an early stress component also for this procedure. However, there was no corresponding elevation of heart rate, indicating that the stress responses were specific to the procedures with imposed stress. The group median SEMG values were below 5% EMG_{max} for all procedures, also indicating a low biomechanical load (Fig. 2A).

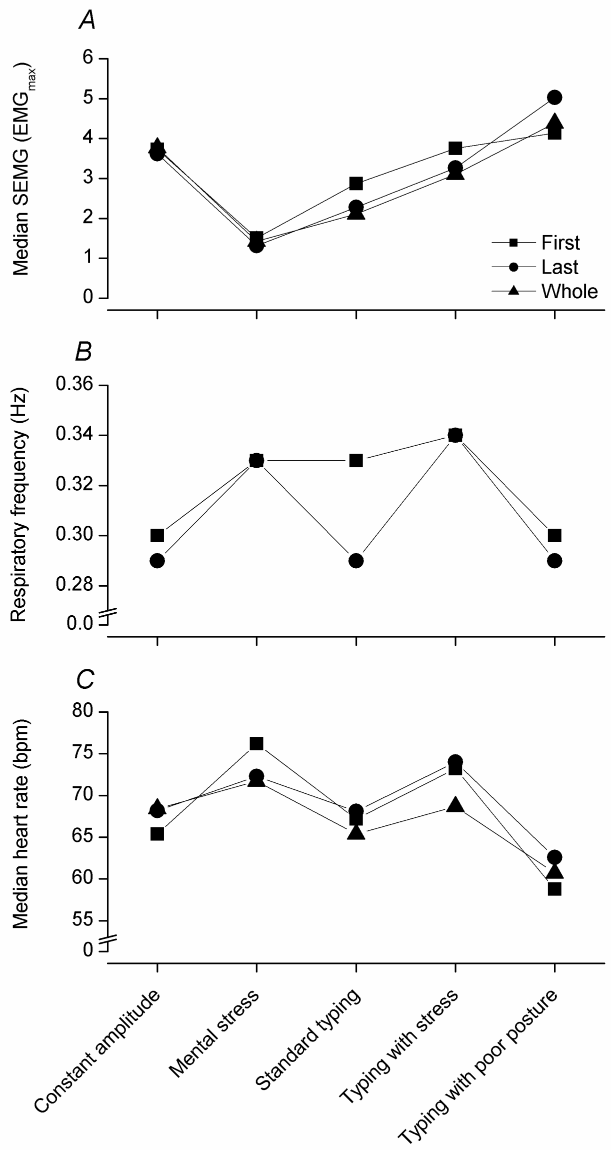

Figure 2. First min, last min and full recording median values of SEMG (*A*), respiratory frequency (*B*) and heart rate (*C*) for five 10-min procedures; constant amplitude contractions (constant amplitude), imposed mental stress with minimal body movement (mental stress), typing in ergonomically good position (standard typing), typing with money reward for improved performance (typing with stress) and typing with elevated table and no arms support (typing with poor posture). Last min values only include motor units recorded for a minimum of 5 min.

Paper I:

Isometric contractions below 10% EMG$_{max}$ of 5, 10 and 30 min duration and with transient voluntary increase in SEMG level were used in this study. An effect of transient force increase is a marked depression in motor unit firing rate observed immediately after the intermittent increase in SEMG (Fig. 3). The firing rate depression outlasted the force increase by up to 10 s.

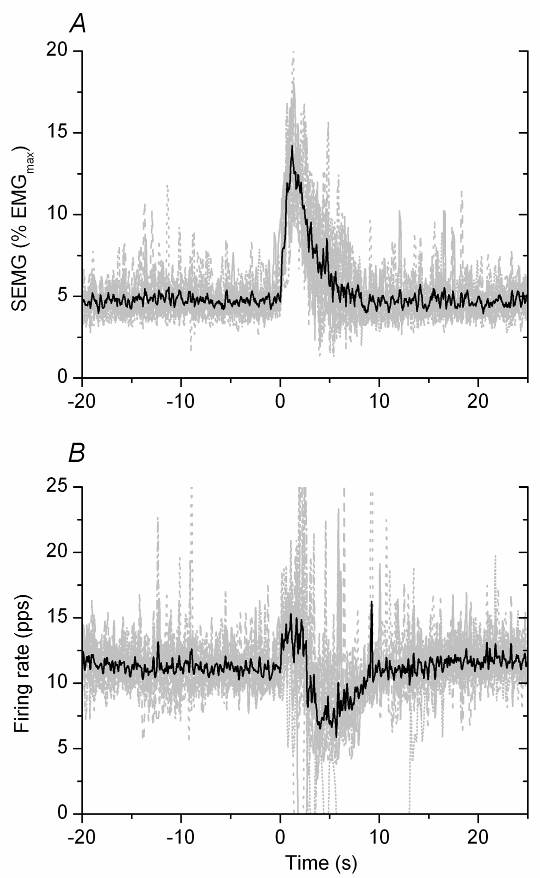

Figure 3. SEMG response (*A*) and motor unit firing pattern (*B*) of 11 consecutive contractions, aligned at start of force increase. *Black line* shows average level. *Gray lines* show motor unit firing pattern for each contraction. Periods of no motor unit activity are excluded when calculating average firing rate. Note the dip in firing rate whilst the SEMG remains at or above the initial level.

Low-threshold motor units recruited at the beginning of long-duration contractions were observed to stop firing while motor units of initially higher threshold stayed active (Fig. 4). Derecruitment of motor units coincided with the end of the EMG pulses. The periods of no firing (silent periods) of derecruited motor units lasted from a few seconds to several minutes. Re-recruitment of motor units was generally linked to planned or small spontaneous EMG pulses.

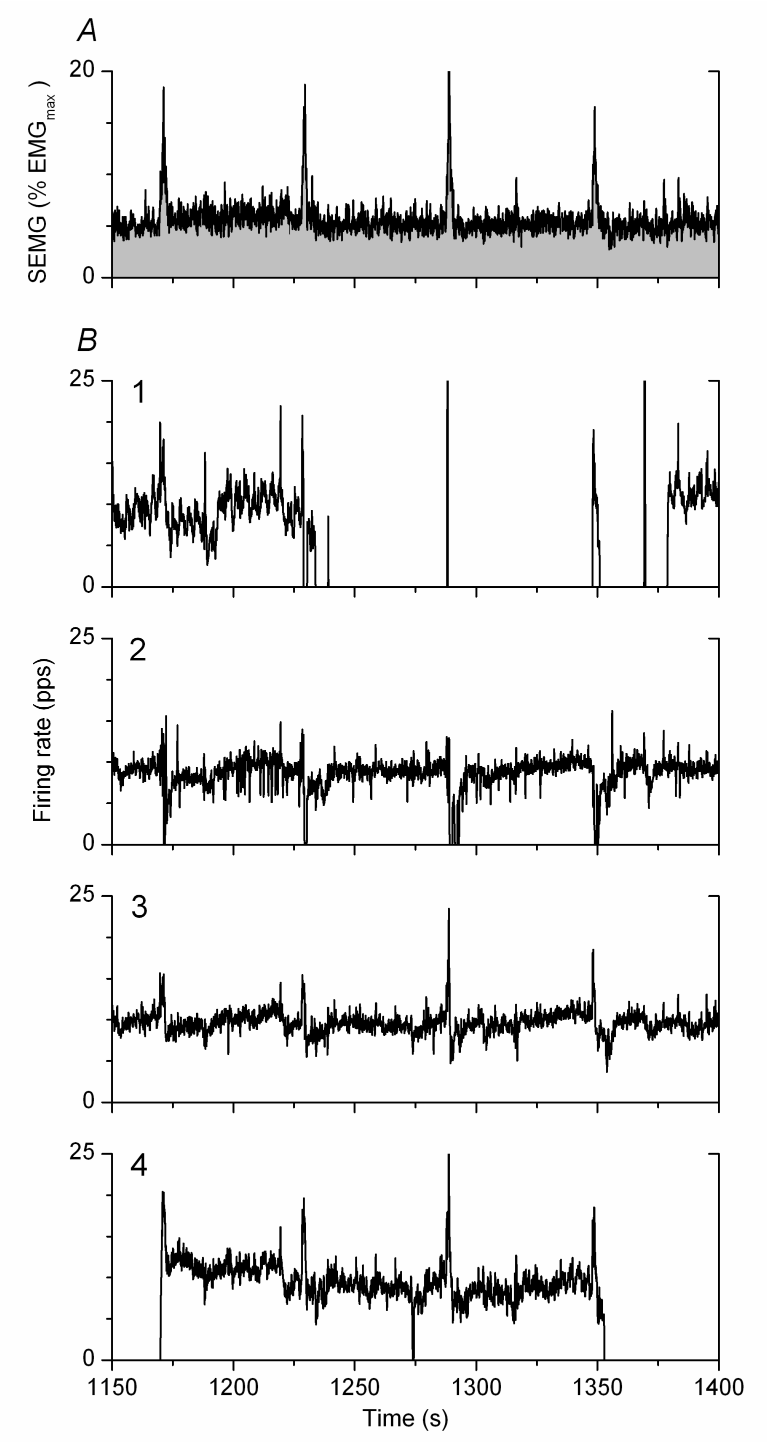

Figure 4. SEMG response (*A*) and firing pattern of 4 motor units (*B*) to show threshold reversal between motor unit 1 and 4.

Paper II:

Motor unit firing behavior in constant-amplitude contractions was compared to firing behavior in vocational activities requiring arm movement. Mental stress was introduced to examine whether this induced higher firing rates than voluntary contraction at similar SEMG amplitude. Sinusoidal and staircase force profiles were used to explore firing behavior in dynamic contractions.

The firing rate in constant amplitude contractions increased from 10.5 to 12.5 pps when SEMG increased from <2% to >4% EMG_{max} (Fig. 5). There was no consistent difference in firing rate between constant amplitude contractions and firing rate in vocational tasks at the same SEMG level. The introduced mental stress did not increase firing rate relative to procedures without mental stress.

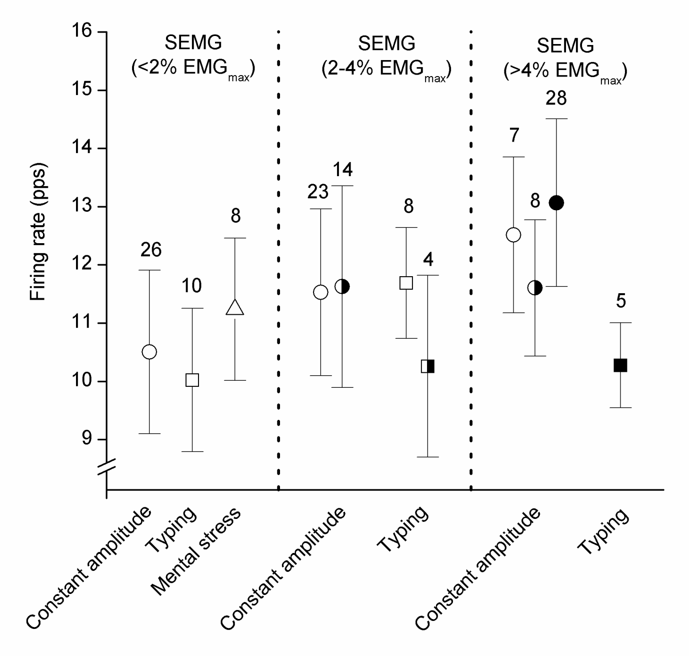

Figure 5. Motor unit firing rate as a function of motor unit recruitment threshold. Motor units were classified by threshold into three groups, <2, 2-4 and >4% EMG_{max}, and firing rates in contractions with SEMG amplitude in the same amplitude ranges determined. *Vertical dashed lines* delineate SEMG amplitude groups. Three main procedures are identified with *circles* (constant amplitude), *squares* (all variations of typing) and *triangles* (mental stress). For each procedure, *open* symbols show mean firing rates with SD of motor units with threshold <2% EMG_{max}, *semi-filled* symbols show mean with SD for motor units with threshold 2-4% EMG_{max} and *filled* symbols mean with SD for motor units with threshold >4% EMG_{max}. The number of motor units contributing to the statistics is indicated above the error bars.

Motor units with different recruitment threshold converged to near identical firing rates after a few min of firing within the same experiment. New motor units were recruited in repeated oscillations of sinusoidal contractions. Finally, bursts of high frequency firing were observed in dynamic contractions.

Paper III:

Materials from other study purposes were used including that from paper II. Spike-triggered averaged (STA) technique was used to examine motor unit potentials and their dependence on contraction amplitude and firing history. Peristimulus time histograms were constructed to estimate motor unit synchrony. The area of STA-derived motor unit potentials showed a strong linear increase for increasing SEMG amplitudes up to 14% EMG_{max} (Fig. 6). Motor units with different recruitment threshold had similar area of the STA-derived potential at the same SEMG amplitude.

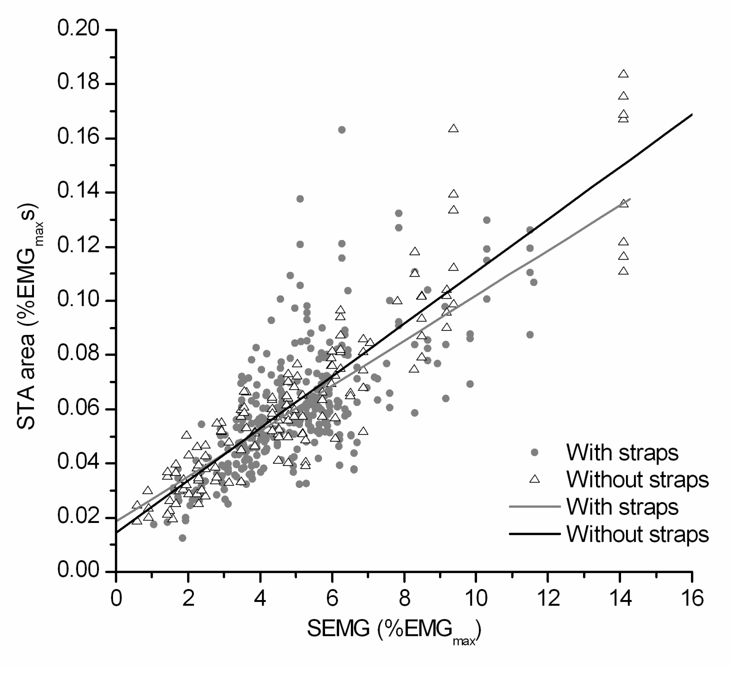

Figure 6. Scatter plot with regression lines of STA area (calibrated as %EMG_{max} s) versus SEMG amplitude of all STA-derived motor unit potentials. Potentials derived from procedures with straps providing resistance to attempted shoulder elevation (*circles* and *grey line*) and contractions without shoulder resistance (*triangles* and *black line*) are shown.

Motor unit synchrony accounted for on average 2.8% additional firings within ±2 ms of the triggering motor unit (Fig. 7). Most of the increase in area with increasing SEMG amplitude seems to be due to motor unit synchrony and should be taken into consideration when applying this method on motor units presenting wide potentials. The area of a motor unit firing alone was ~0.02% EMG_{max} s. The mean STA-derived area at 5% EMG_{max} was 0.06 % EMG_{max} s. With 2.8% synchronization of firings, ~70 motor units are required to compensate for this increase. Simulation experiments indicate a 17-fold increase in average rectified value (ARV) from 1 motor unit firing at 10 pps to 70 motor units firing at 12.5 pps. The SEMG amplitude generated by 70 motor units is ~3.4% EMG_{max}. If the average area of the 10 lowest threshold motor units is used, the equivalent SEMG amplitude is ~4.4% EMG_{max}.

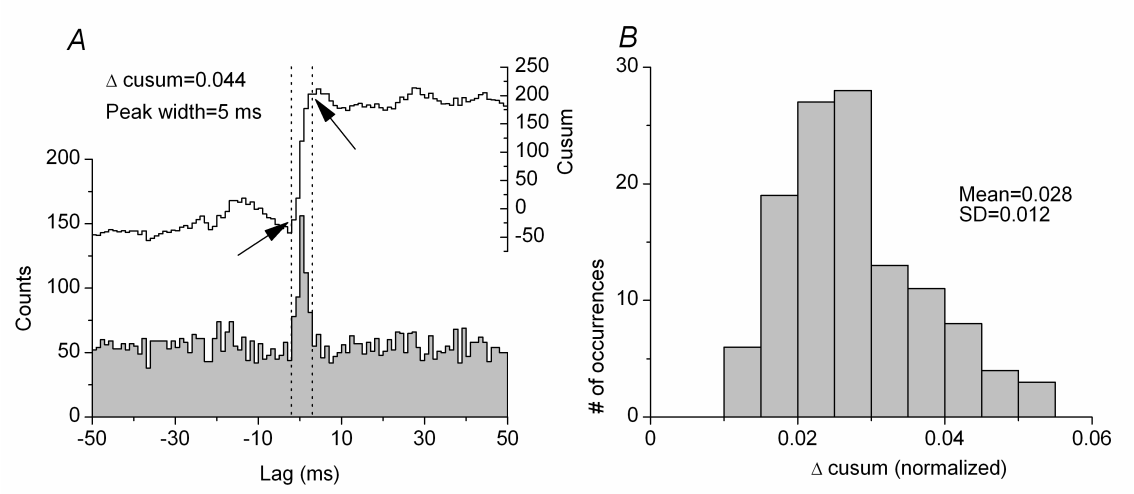

Figure 7. Peristimulus time histogram of motor unit firings (*A*) and normalized amplitude of region with elevated probability of firing in the PSTH histograms, quantified by the cumulative sum (cusum) technique determined from the inflections of the cusum plot above the PSTH histogram (*B*).

Paper IV:

Materials from previous studies were analyzed for frequency content of firing rate modulation. Mean firing rate was close to 10 pps with respiratory modulation of peak firing rates ranging from 2-5 pps for continuously firing motor units. All motor units in low-level (2-3 %EMG_{max}), constant-amplitude contractions showed respiratory modulation of firing rates at ~0.30 - 0.35 Hz (Fig. 8A, and cf. Fig. 2B for median respiratory frequency). Motor units near recruitment threshold showed strong modulation. At higher contraction amplitudes the modulation was reduced. Respiratory modulation of firing rates was much reduced in experiments with mental stress and typing even for low SEMG amplitudes. Firing rate modulation at double the respiratory frequency was observed for some motor units (Fig. 8B). For most motor units the peak firing rates were observed midway between inspiration and expiration, but motor units with peak firing between expiration and inspiration were common. Figure 8A is an example of an experiment where both phases were simultaneously recorded. No motor units were firing in phase with expiration.

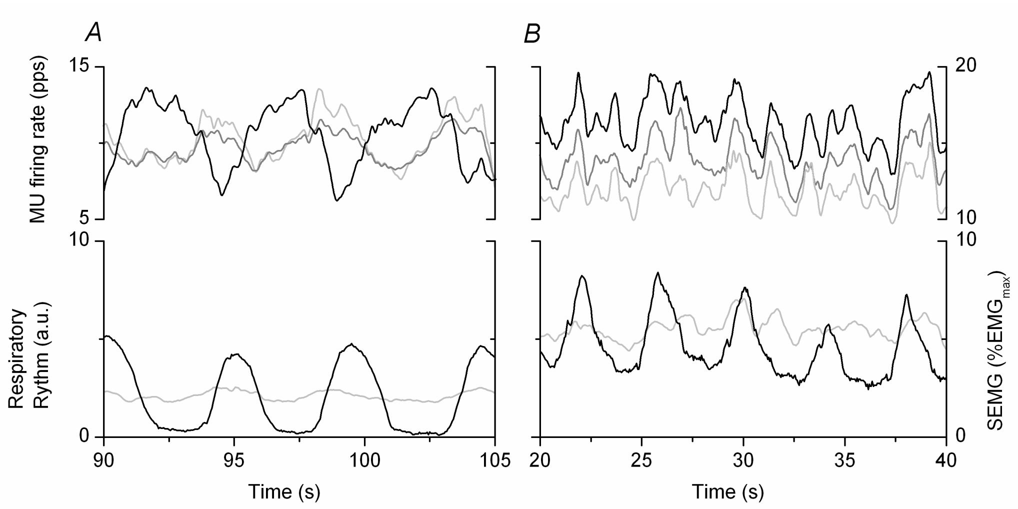

Figure 8. Time plots of firing rate modulation of three motor units (top traces) and upper chest movement with SEMG amplitude (bottom traces, *black* and *grey lines* respectively). Selected time periods of two constant amplitude contractions (*A* and *B*) show specific features in firing pattern; opposite phase modulation of motor unit firing was observed (*A*), as was firing rates of double the respiratory frequency (*B*).

Discussion

This thesis provides a comprehensive description of trapezius motor unit firing behavior in low-amplitude contractions. The experiments have pointed out control strategies of the trapezius as a postural muscle that differ from extremity muscles. Attention is given to motor unit recruitment threshold as a reference for firing behavior. Furthermore, a novel respiratory modulated input to trapezius motoneurones has been characterized. The results are discussed with reference to the hypothesis of motor unit over-exertion causing development of shoulder- and neck pain.

Adaptations to postural functionality

Muscles with a postural function have a high proportion of type I muscle fibers, e.g., around 87% for the soleus muscle (Johnson *et al.*, 1973). In the same study, approximately 54% of the fibers in the trapezius were found to be of type I. Another study found that the fraction of type I fibers was 58% for females and 67% for men in the descending I portion of the trapezius, close to the recording site used in our study, (Lindman *et al.*, 1990; Lindman *et al.*, 1991). In soleus, FDI and triceps brachii, the innervation ratio of type I fibers is smaller than of type II fibers (Enoka & Fuglevand, 2001). FDI type I muscle fibers comprise approximately 50% of the fibers (Dennett & Fry, 1988) while the motoneuron pool innervates 84% type I fibers. If this skewed distribution is valid also for other muscles, and with type I fibers comprising over 50% of the muscle fibers in the trapezius, most, if not all fibers activated below 10% EMG_{max} in the trapezius are type I.

STA derived motor unit potentials extracted from trapezius motor units recruited below 10% $EMG_{max,}$ indicate they are of similar size, regardless of recruitment threshold (paper III). This is assuming the size of surface action potentials is correlated with the size of the motor units and that the STA-derived potentials are representative for the source potentials. Simulation experiments, based on the estimated initial size of motor units from our recording site, and firing rates at different contraction amplitudes, determined in paper II, indicate that many motor units are active at low contraction amplitudes. In paper III it is estimated that approximately 70 motor units are active at 5% EMG_{max}. The firing rates stay low in sustained contractions. There is only a small increase in mean firing rate from ~10 to ~12 pps in contractions with SEMG amplitude increasing from <2 to >4% EMG_{max} (paper II). Thus, the main contribution to force increase in the trapezius in slowly augmenting contractions is recruitment of new motor units. Taken together, these motor control features are likely adaptations to sustain the long-duration, low-amplitude contractions of the trapezius muscle.

Trapezius motor unit firing rate features

Previous studies have described a number of features regarding trapezius motoneuron firing behavior. The typical firing rate of trapezius motor units recruited at low contraction amplitudes is 10-15 pps. The onset of motor unit firing in constant amplitude contractions is often characterized by an initially higher firing rate that is slightly reduced to a stable firing rate. The firing rate is relatively stable in constant amplitude contractions and with little, if any, firing rate modulation in response to slow (<3% EMG_{max}/s) variations in force (Westgaard & De Luca, 2001). However, trapezius motor units are sensitive to abrupt changes in the SEMG level. In staircase contractions when the contraction amplitude is abruptly increased by ~2% EMG_{max}, motor units increases firing rates slightly. If the new level is maintained the firing rate stabilize at a moderately higher level. Motor units that are recruited at the abrupt force increase have higher firing rates than active units, but all motor units converge to a common firing rate during the static part of the contraction profile (paper II). A relatively high modulation of firing rates (5-10 pps) is also observed in sinusoidal contractions tracing changes in SEMG with 6s cycles, corresponding to contraction rate of change of ~3% EMG_{max}/s (paper II). In ramp contractions with rates of rise 1% EMG_{max} /s or more, trapezius motor units show increasing firing rate modulations with early recruited motor units showing higher firing rates than later recruited units, resembling the onion skin pattern observed for distal muscles such as the FDI (Westgaard & De Luca, 1999). Transient *decreases* in SEMG during constant amplitude contractions may be accompanied by a transient decrease in motor unit firing rate, and in some situations silencing of motor units (Westgaard & De Luca, 1999). When new motor units are recruited, a short dip in the firing rate of some, but not all, of the already active units are often observed. This indicates a reciprocal inhibition or some form of competitive interaction that affects selected motor units.

Post-pulse depressions of firing rate

A consistent observation of motor unit firing behavior in the present study was the depression of firing rate immediately after a voluntary brief *increase* in SEMG amplitude. The depression of firing rate outlasted the activity-time of new motor units recruited during the EMG pulses by several seconds and was maintained on average 4 s after the SEMG were back at pre-pulse level. The slow recovery of pre-pulse firing rate level suggests that a mechanism other than the all-or-nothing behavior of plateau potentials is involved. A slow withdrawal of inhibitory activity could partially explain the slow recovery of firing rate. Recurrent inhibition by Renshaw cells is a system that acts strongest on low-threshold motor units (Hultborn *et al.*, 1988) and is more developed in proximal than distal muscles (Katz *et al.*, 1993). The effect of recurrent inhibition is a reduction of net excitatory input to motoneurones and thereby sudden increase in firing rate. Once the firing rate is reduced, the effect of the inhibition is also reduced. However, inhibitory systems are not known to be effective for many seconds in a manner that can explain the post-pulse depression of firing rate alone. Altered membrane properties and prolonged afterhyperpolarization would increase the interspike interval, thereby decreasing the probability of firing in response to a constant excitatory drive. At present the cause of the prolonged depression in post-pulse firing rate is unknown.

During the SEMG pulse many additional motor units are recruited that can potentially exert inhibitory action on already active motor units through e.g. the Renshaw system. In some situations, low threshold motor units are completely silenced when new motor units (with higher recruitment threshold) are recruited during the EMG pulse. In paper I it is shown that motor unit silent periods are induced by transient *increases* in SEMG. Thus, force variation in either direction seems to promote silent periods.

Inactivation of plateau potentials was suggested as a contributor to silent periods (cf paper I). The sudden increase in firing rate during the transient force increase may initiate a strong Renshaw inhibition that could act as off-stimuli for plateau potentials, effectively increasing their activation threshold and thus completely silence motor units that are close to recruitment threshold. Conversely, re-activation of plateau potentials may reduce motor unit threshold to below the static contraction level and trigger re-

recruitment of the same motor unit. Motor units with recruitment threshold close to the static SEMG activity level have longer and more frequent periods of inactivity than the lowest threshold motor units. Conceivably, if the recruitment threshold relative to the static EMG level is too large, the motor unit is not silenced by inactivation of plateau potentials. The silencing of motor units generally occurred 3 min or later into the experiments, which indicates there is also a dependence upon firing history.

Trapezius firing pattern compared to extremity muscles

The control scheme governing a muscle is in general adapted to the motor tasks the muscle frequently participate in (Loeb, 1985). The firing pattern of the trapezius muscle differs from the firing pattern of extremity muscles in several ways. There is a tendency for greater rate modulation in distal than in proximal muscles and new motor units are recruited over a larger force range in proximal than distal extremity muscles (Kukulka & Clamann, 1981; De Luca *et al.*, 1982). It has been shown that the firing behavior of the FDI during isometric and slowly augmenting contractions (force-rate of increase up to 1% EMG_{max} /s) is sensitive to changes in the muscle activity and with lower firing rate of new motor units compared to already active units (Westgaard & De Luca, 2001). In trapezoidal contractions the firing pattern of FDI motor units with different recruitment threshold resembles the appearance of an onion skin pattern, i.e. the lowest threshold motor units consistently exhibit the highest firing rates (De Luca *et al.*, 1982). If this was the case also during the sustained activity patterns of the trapezius muscle, the risk of motor unit over-exertion and damage would be higher.

The trapezius, as a postural muscle, must be able to tolerate sustained long duration contractions (Jensen *et al.*, 1993a; Thorn *et al.*, 2002), whereas the FDI is normally involved in short-duration phasic contractions in fine control of the fingers. The differences in the motor control schemes between the trapezius and FDI in low-amplitude contractions may thus indicate an adaptation to the functional tasks of different muscle groups.

Motor input to upper trapezius

The motor input to the trapezius muscle derives from the spinal accessory nerve (XI) (Weisberger, 1987) but also from the cervical plexus (Kierner *et al.*, 2001). It appears that the upper trapezius is predominantly innervated by the spinal accessory nerve, while the lower parts may receive its innervation from the cervical segments of the spinal cord. The spinal accessory nerve is part of the special visceral motor column together with the motoneurones contributing to the glossopharyngeal (IX) and vagus (X) nerves, constituting the nucleus ambiguus. The preganglionic autonomic neurons of the nucleus ambiguus, located in the medulla, innervate among other organs the esophagus, heart and the respiratory system. The glossopharyngeal and vagus cranial nerves are predominantly autonomic mixed nerves containing both sensory and motor nerves. They transmit sensation from the pharynx and upper airways and taste from parts of the tongue and oral cavity. The glossopharyngeal nerve transmits information from the neck such as e.g. blood pressure and blood oxygen saturation, while the vagus nerve transmits information from the respiratory, cardiovascular and gastrointestinal organs. The parasympathetic motor fibers of the glossopharyngeal nerve innervate the parotid salivary gland and the stylopharyngeus which raises and dilates the pharynx. The rest of the voluntary muscles of larynx and pharynx are controlled by the vagus nerve. This nerve also innervates internal organs of the neck, thorax and abdomen (Kandel *et al.*, 2000).

The spinal accessory nerve thus shares a common developmental origin and relatively close location in the brainstem and medulla with cranial nerves that, among other organs, control the respiratory system. This may be of importance when considering the respiratory modulation seen in trapezius motor units.

The noradrenergic locus coeruleus (LC) neurons are activated by (but not limited to) stressful stimuli. In monkeys LC activity was found to correlate with performance in visual discrimination tasks requiring focused attention (Aston-Jones *et al.*, 1999). Neurons from the LC project to broad areas of the CNS, such as cerebral cortex, cerebellum and brain stem, and with rich innervation of the spinal cord motoneurones in some species (Holstege & Kuypers, 1987). The neurons show extensive collateralization where each terminal axon forms multiple presynaptic varicosities that may act in a diffuse hormone-like manner (Stricker & Zigmond, 1986). Other noradrenergic neurons originating from lateral tegmentum and dorsal medullary cell groups innervate, among other regions, primary motor and visceral nuclei in the brain stem. These innervations include cranial nuclei such as motor trigeminal, fascial, hypoglossal, vagus and nucleus ambiguus (Björklund & Lindvall, 1986). These nuclei have no LC innervations. In rats noradrenergic fibers in the spinal cord are found in specific regions of the spinal grey matter, including around large motoneurones in the ventral horn especially at cervical and lumbar levels (Westlund *et al.*, 1983). LC neurons facilitate motoneuron activity (Chan *et al.*, 1986) and may thus be at least partially responsible for stress-related activity observed in trapezius motoneurones.

The relevance to development of shoulder- and neck pain

Contraction level from 1-10% EMG_{max} is representative for the range of trapezius activity in common daily living (Westgaard *et al.*, 2001; Holte & Westgaard, 2002). Changes in trapezius muscle morphology, especially in the descending portion of the trapezius, have been attributed to work-related exposures (Lindman *et al.*, 1990). These changes include enlarged type I fibers as well as ragged red fibers and moth-eaten fibers (with dysfunctional mitochondria) thought to result from prolonged and repetitive use of low-threshold motor units.

In the present thesis it is shown that many motor units are active at low-amplitude contractions (i.e. <10% EMG_{max}). It is furthermore argued that these motor units are of similar size based on the STA derived potential. It is also known that recruitment of new motor units depress the firing rate of already active motor units (Westgaard & De Luca, 2001). Thus, moderate variation in contraction amplitude could easily bring about changes in the activity pattern of motor units in the trapezius, in particular, brief increases in force promote silencing / substitution of motor units. However, it seems that if the recruitment threshold of a motor unit is much lower than the static activity level (in the order of ~5% EMG_{max}), silencing of motor units are less likely to occur. Silencing and substitution of motor units have been proposed as a mechanism to protect motor units from excessive fatigue during low-amplitude sustained contractions (Person, 1974).

The trapezius shows strong response to mental stress in terms of elevated SEMG amplitude and recruitment of motor units (Wærsted & Westgaard, 1996). Elevation of heart rate was used as an indicator of successfully induced mental stress (paper II). In the mental stress procedures the heart rate was increased by ~5 beats per min. There was no significant increase in firing rate in procedures with induced mental stress compared to procedures without mental stress at the same SEMG level. In the two-choice reaction test there was virtually no biomechanical requirement for motor unit activity, yet for several subjects many motor units were firing during the 10 min time course of this procedure. It is likely that a number of these motor units were recruited by the induced mental stress factor. Thus, even though the mean firing rate did not increase, mental stress has the effect of recruiting additional motor units. The different respiratory modulation led to the argument that stress may activate another excitatory input different from that operating in voluntary contractions. The noradrenergic pathways are a candidate system to modulate trapezius firing behavior during stress. Trapezius motor units normally have stable mean firing rates in static and slowly augmenting contractions with rate of rise <3% EMG_{max} /s. The added load of stress-induced motor units increases the overall SEMG level thereby increasing the distance between the recruitment threshold and SEMG level of the lowest threshold motor units (SEMG level representing the net excitatory input to the motoneuron pool). When the difference between recruitment threshold and the SEMG-level increases, the excitatory drive to the motor units outweighs the inhibitory mechanisms that could potentially silence their activity. The effect of stress could thus be less frequently occurring silent periods of the lowest threshold motor units.

Respiratory modulation of firing rate is much reduced in motor units in the mental stress experiments despite low contraction amplitude. In paper IV, it is suggested that the attenuation of the firing rate modulation is due to a state change in the segmental cord inter-neuronal network that includes suppression of the Renshaw system of interneurons; i.e., LC noradrenergic neurons are shown to have this effect (Fung *et al.*, 1987). Consequently, the inhibitory action that could trigger substitution or silent periods of motor units may be less frequently occurring.

Unresolved issue; SEMG power vs. intramuscular EMG activity

In paper I it was consistently observed that the firing rate of motor units showed a depression in firing rate in the down-phase of, and for a short period immediately after transient EMG pulses, despite the fact that the SEMG-level was higher than, or equal to the SEMG-level before the transient increase (cf. Fig. 3). The depression of firing rate while SEMG is back at pre-pulse level therefore constitutes a mismatch between motor unit activity and the simultaneously recorded SEMG amplitude.

This mismatch could be due to “fill-in” by other motor units detected by the much larger detection territory of the SEMG electrode compared to the relatively few motor units detected by the intramuscular electrodes. However, a fill-in motor unit was never detected in any of the instances where this could apply. Such selection bias with the intramuscular electrode is possible but seems less likely as the explanation for the mismatch in signal powers.

Increase in membrane potential due to increase in Na^+-K^+ -pump activity during the post-pulse period could explain the mismatch in signal power since each motor unit would then contribute more to the SEMG signal. Transient enlargement of the M-wave, termed pseudofacilitation, has been demonstrated in human voluntary 3 s isometric contractions of the abductor pollicis brevis (McComas *et al.*, 1994). In the same study (but in biceps brachii under ischemic conditions) it was found that the enlargement commenced after ~2 s when the motor point was stimulated at 10 Hz. In these experiments the enlarged potentials gradually returned to initial size over several min after cessation of the stimuli.

Another possibility is changes in motor unit synchronization and amplitude cancellation effects. In simulation experiment amplitude cancellation reached 85% using 40 motor units (paper III). The number of motor units and the duration of the action potential is the most profound factors influencing amplitude cancellation (Keenan *et al.*, 2005), the trapezius motor units presenting relatively wide potentials. In simulation experiments motor unit synchronization increases EMG amplitude and decreases force steadiness (Yao *et al.*, 2000), although not to a major extent (Keenan *et al.*, 2005). Conceivably, the SEMG amplitude could show increased amplitude caused by synchronous firing of motor units. STA derived motor unit potentials immediately before and after the SEMG pulse showed no change in the area of the potentials. However, the triggering motor unit may contribute as little as 25-30% of the STA-derived potential (paper III) which lowers the sensitivity to detect changes in the source potential.

Since the subjects control their force output based on feedback of the RMS-detected signal, enlargement of the surface representations of the motor units, whether caused by enlarged membrane potential or due to less cancellation, would maintain the SEMG-level while firing rate decreases. If so, the true representation of the net excitatory drive would be reflected by the firing rate of the motor units, rather than the SEMG-level.

The possible reasons for the mismatch in signal power is at present only speculations as our data cannot be used to falsify or confirm the above mentioned factors.

Conclusion

The results presented in this thesis characterize trapezius motor unit firing behavior in sustained, long-duration, low-amplitude contractions typical of daily living. It appears this muscle has control strategies to cope with different types of activity patterns, and has anatomical features well adapted to meet postural demands.

Anatomy

Trapezius muscle fibers active below 10% EMG_{max} are indicated to be of similar size, thus deviating from the Henneman size principle. A relatively high number of motor units seem to be active at low contraction amplitudes (~70 motor units at 5% EMG_{max}). This organization may be advantageous to be able to sustain contractions of long duration, typical of postural functionality.

Firing pattern

The firing behavior of the trapezius in sustained, long-duration, low-amplitude contractions is characterized by relatively invariant firing rates to different motor tasks, with only a small increase in firing rate in response to increased contraction amplitude. Motor unit silent periods and threshold reversals are frequently occurring. These findings supplement previous studies that suggest a different control strategy in the trapezius muscle compared to extremity muscles in sustained low-amplitude contractions.

There is strong modulation of firing rates in response to dynamic contractions, suggesting a control strategy resembling that of extremity muscles. The trapezius may thus adopt control strategies depending on specific demands.

The voluntary excitatory drive to trapezius motor units appears to be modulated by autonomic input, with respiratory modulation of the firing rate observed in low-amplitude contractions. The modulation was attenuated in experiments with induced mental stress indicating at least two different sources of pre-motor input. The additional pre-motor input from autonomic pathways contributes to the excitatory input to trapezius motoneurones, thereby facilitating low-threshold trapezius motor unit activity.

Relevance to pain development

In a pain development perspective, the most important variables to investigate in relation to over-exertion of single motor units seems to be the duration and pattern of substitution and silent periods. An earlier publication showed substitution of motor units in response to brief reductions in excitatory drive. In the present thesis it was shown that brief *increase* in force promotes motor unit silent periods and threshold reversals between motor units in long-duration, low-amplitude contractions. Thus, motor unit substitution can be induced by force variation in either direction.

Motor units with recruitment threshold near the static SEMG-level presented the longest silent periods. It appears that as the distance between recruitment threshold and the static SEMG-level increases, the frequency and duration of silent periods decreases.

STA method

This thesis also address important methodological considerations when applying STA to quantify motor unit size, the number of motor units and muscle fiber conduction velocity, especially for motor units presenting wide action potentials. The STA-derived motor unit potentials show a 4-fold increase with increasing contraction level for motor units recruited below 10% EMG_{max}. This increase is to a major extent attributed to motor unit synchronization. Thus, due caution should be employed when comparing the size of STA-derived potentials extracted at different contraction amplitudes.

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Paper IV

Manuscript

Respiratory and stress-induced activation of low-threshold motor units in the human trapezius muscle

by

Westgaard RH, Bonato P & Westad C

Respiratory and stress-induced activation of low-threshold motor units in the human trapezius muscle

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ABSTRACT

The study aimed to characterize respiratory modulation of trapezius motor unit firing in low-amplitude contractions. Feedback-controlled, constant amplitude contractions with shoulder elevation, tasks with mental stress and typing were performed, representing presumed different activation modes of trapezius motoneurons. Single motor unit activity was recorded by a quadrifilar fine-wire electrode. A surface electrode simultaneously recorded surface electromyographic (SEMG) activity. Contraction amplitudes ranged from 1 to 10% of the SEMG signal at maximal voluntary contraction (1-10% EMG_{max}). All motor units recorded in low-amplitude (<3% EMG_{max}) contractions with shoulder elevation showed firing rate modulation (2-5 pulses per s) at the respiratory frequency. Respiratory firing rate modulation was markedly reduced or not observed in constant amplitude contractions >3% EMG_{max}. Respiratory modulation was not evident in the SEMG signal, presumably because respiratory phase varied between motor units. Most motor units had peak firing rates at the transition from inspiration to expiration, but peak firing rate at the transition from expiration to inspiration or at the first harmonic frequency was also observed. Respiratory modulation of firing rates was significantly reduced for same contraction amplitude in experiments with mental stress. We conclude that both central respiratory drive and peripheral afferent input may contribute to the respiratory modulation of firing rates; however, respiratory modulation of lumbar motoneurons in paralyzed cats points to a central source of the excitatory input. We suggest the reduction in respiratory modulation of motor activity with mental stress is due to a state change in the interneuron network of the trapezius motor nuclei.

INTRODUCTION

In the recent past, there has been considerable interest in the motor control of the trapezius (e.g., Thorn et al. 2002; Zennaro et al. 2003), motivated by musculoskeletal pain research since shoulder pain often is located to this muscle (Simons et al. 1999). Ergonomic and occupational research has focused on the biomechanical role of upper trapezius as a lifter and stabilizer of the scapula, providing a platform for arm movement (e.g., Vasseljen and Westgaard 1997; Jensen et al. 1998). However, trapezius contributes in motor tasks other than arm movement: it participates in the control of head posture and is described as an auxiliary respiratory muscle with a habitual inspiratory activity pattern among chest breathers (Schleifer et al. 2002). Chest breathing implies that intrapleural inspiratory pressure is generated with a significant contribution from expansion of the upper rib cage. The trapezius is innervated by the spinal accessory (XI) nerve. Trapezius motoneurons derive from the special visceral group of neurons together with, among others, motoneurons of the facial motor nuclei and nucleus ambiguus, innervating laryngeal and pharyngeal muscles (Kandel et al. 1991).

There is controversy regarding the role of trapezius as an auxiliary respiratory muscle. Most texts on respiratory control present trapezius as a muscle with an inspiratory function on the basis of functional anatomy considerations, i.e., upward expansion of the rib cage through a lifting action on the clavicle (Campbell 1968; Zemlin 1997). This function is, however, poorly documented by objective measures in normal breathing. Other researchers disregard a respiratory action of trapezius (Basmajian and De Luca 1985). It was recently shown that trapezius has a clear expiratory function in demanding vocalization tasks such as operatic singing (Pettersen and Westgaard 2004).

Low-level, spontaneous trapezius muscle activity is often observed in nominal resting situations, indicating that the membrane potential of low-threshold motoneurons is maintained close to threshold also in situations without purposeful shoulder or head movement. Trapezius motor activity tends to increase in situations with stress and can be difficult to silence except by use of biofeedback measures (Bansevicius et al. 1997). In previous studies we have documented motor control features of trapezius that distinguish this muscle from extremity muscles (Westgaard and De Luca 2001; Westad et al. 2003; Westad et al. 2004). The aim of the present study is improved insight into pathways that provide excitatory input to trapezius motoneurons at low contraction levels, with emphasis on respiratory modulated and stress-induced muscle activity. The frequency content of trapezius motor unit activity patterns in quiet breathing is described. Respiratory modulation of firing rates is observed during low-amplitude contractions and is characterized in relation to respiratory phasing, contraction amplitude and motor task.

MATERIAL AND METHODS

Motor unit recordings collected for other study purposes were analyzed for frequency content of firing rate modulation (Westad et al. 2003; Westad et al. 2004). This includes trapezius electromyographic recordings from 20 healthy subjects, 9 males and 11 females, age ranging from 20 to 57 yrs. Experimental procedures and methods are described in detail in the above listed papers; here the description of methods and procedures is limited to those aspects relevant for the present study. The experiments were carried out according to the Declaration of Helsinki. Each subject read and signed an informed consent form approved by the local Institutional Review Board prior to participating in the study.

Experimental procedures

Simultaneous electromyographic recordings by surface and intramuscular electrodes were performed. The SEMG signal was calibrated in technical units (µV) and in percent of the RMS-detected EMG activity at maximal voluntary contraction (%EMG_{max}).

Feedback-controlled, constant amplitude contractions of two to 30 min duration were analyzed. All feedback-controlled contractions were carried out with the subject seated and straps placed over the shoulders to provide resistance to the attempted movement of elevating the shoulders. Shoulder elevation was performed bilaterally, with EMG data collected from the left trapezius. Contractions were carried out at low amplitude (typically ~2% EMG_{max} of 2 min duration) to observe motor unit firing patterns near threshold for recruitment and at higher amplitude (typically 4-6% EMG_{max} of 10 min duration) to observe firing patterns in sustained contractions with stable firing. The contraction amplitude was determined in brief trial contractions to ensure that a suitable number of motor units with stable firing could be recorded. Motor tasks mimicking muscle activation in daily living, such as motor activity in typing and trapezius motor response to mental stress were also carried out (Westad et al. 2004). Contraction amplitudes varied from 1 to >10% EMG_{max}, the higher amplitudes were observed in typing tasks and were maintained for periods up to a few minutes duration. In the experiments focusing motor response to mental stress, the subject was placed in front of a personal computer (PC) to perform a complex, attention-demanding reaction test presented on the computer (Westgaard and Bjørklund 1987). Elbows rested on the tabletop and the only body movement required was finger pressure on one of two buttons. The execution of the test was self-paced, but the subject first carried out the test for 2 min at a steady pace while attempting to maintain a low failure rate. In the recorded experiment a small monetary reward was offered if the subject performed 10% faster without increasing the failure rate. Feedback provided on the computer screen informed the subjects about response speed and whether the answer was correct (Wærsted et al. 1994); at the same time a new task was presented on the screen. For the typing procedure the subjects were asked to type a text, placed next to the monitor, using a standard keyboard. Three variations of the task were performed: with optimal posture and no pressure on performance, with optimal posture and pressure on performance (i.e., a small monetary reward if the subject performed 10% better in terms of number of words and with the same or fewer errors than in the first task), and finally with elevated table top to provoke typing at higher muscle activation levels. All trials were of 10 min duration with minimum 2 min rest between each trial.

Physiological recordings and analyses

The SEMG signal was detected by an active differential electrode with two circular recording surfaces (6 mm in diameter, 20 mm inter-electrode distance). The electrode was positioned with the medial recording surface 20 mm lateral to the midpoint of a line between the C7 spinous process and the acromion (Jensen et al. 1993). The SEMG signal was band-pass filtered at 10-1000 Hz, sampled at 2 kHz and the RMS-value digitally estimated over 200 ms non-overlapping windows. The intramuscular EMG signal was recorded with quadrifilar wire electrodes, constructed by placing four 50-μm nylon coated nickel-chrome alloy wires ("Stablohm 800A", California Fine Wire Co, Grover Beach, CA) in a 27-gauge needle. The wire bundle was bent backward at the needle tip to form a hook and was cut transversely approximately one millimeter from the bend, exposing only the cross-section of the wires. The needle was inserted to a depth of approximately 10 mm at a location about 10 mm medial to the midpoint of a line between the C7 spinous process and the acromion, along the direction of the muscle fibers in the area under the SEMG electrode. The needle was removed and the wire bundle remained lodged in the muscle. Three pairs were chosen as the differential input to the amplifiers. The signals were band-pass filtered from 1 to 10 kHz. All EMG signals were stored on a digital recorder (DATaRec-A160, Racal-Heim Systems GmbH, Bergisch Gladbach, Germany). The signals were subsequently reconverted to an analog form and digitized at a sampling rate of 50 kHz on a PC.

The intramuscular EMG signals were resolved into individual motor unit firing trains using the Precision Decomposition technique (LeFever and De Luca 1982; De Luca and Adam 1999). This technique uses template matching, template updating, firing probabilities and superposition resolution to identify the individual firing times of the motor units (Mambrito and De Luca 1984). The instantaneous firing rates of the motor units were obtained by inverting the time series of the inter-pulse intervals.

Respiratory movement was detected by a strain gauge sensor positioned around the upper thoracic wall and sensing chest circumference. Electrocardiographic (ECG) activity was recorded by silver/silver chloride electrodes (Neuroline, Medicotest A/S, Denmark) placed in standard positions across the chest. Respiratory movement and ECG were continuously recorded (Physiometer PHY-400, Premed, Norway) and stored on a palmtop computer (HP 200LX, Hewlett-Packard, USA). The intervals between the threshold-detected R peaks (RR intervals) in the QRS complex were derived on a beat-by-beat basis and the instantaneous heart rate determined by inverting the inter-beat intervals. In three of the experiments respiratory movement and heart rate were synchronized with EMG recordings by also feeding these signals to the digital recorder used to record the EMG signals.

Frequency analysis of motor unit firing pattern

In order to study the frequency content of the firing rate of the detected motor units, we first transformed the unevenly-sampled time series derived from the firing of the motor units in an evenly-sampled time series at 10 Hz by using cubic splines. The frequency content of the motor unit firing rate time series was then studied. Detrending and removal of the mean value (i.e., DC component) were applied before estimating the frequency content of the time series. To avoid that very low frequency components (below 0.02 Hz) could mask rhythmic behaviors associated with motor unit firing rate modulation at higher frequency, a Chebyshev (type II) high-pass filter with transition band 0.005-0.02 Hz, 20 dB of attenuation in the stopband, and 2 dB maximum loss in the passband was designed. The corresponding IIR filter was implemented using a bilinear transformation (with prewarping) and applied to the motor unit firing rate time series as a non-causal filter (i.e., after filtering in the forward direction, the series was reversed and filtered again). Frequency analysis was performed to evaluate the characteristics of the motor unit firing rate time series up to 4 Hz as well as only for the low frequency components, i.e. limiting the analysis to 1 Hz. In the latter case, the time series were downsampled from 10 to 3 Hz (an antialiasing filter was applied).

The time-frequency representation of the motor unit firing rate time series was estimated by using a rectangular window of 30 s, shifting it by steps of 1 s, and estimating the frequency content of each epoch via an autoregressive technique. The

Burg method was used because it is known to provide stable estimates of the frequency content of a time series, is capable of resolving closely spaced rhythmic components, and it can be applied to relatively short time series without compromising the frequency resolution of the output (Proakis and Manolakis 1996). The Akaike technique (Marple 1987) was utilized to select the order of the model for each epoch.

In addition to the time-frequency representation of motor unit firing rate time series, we studied their overall frequency content. The latter was estimated as the frequency marginal of the time-frequency representation, namely by integrating the time-frequency representation along the time axis. This technique is comparable with that of estimating the frequency content of the motor unit firing rate time series over the entire time interval of observation or using a fast Fourier transformation approach (Proakis and Manolakis 1996). However, the technique that we used in this study allowed us to appreciate both the changes in frequency content of the time series over time as well as the overall frequency components contributing to the time series.

A similar technique was utilized to analyze the frequency content of the time series of the RMS values of the SEMG signal. Filtering and frequency transformations were designed consistently to that done for the motor unit firing rate time series, thus facilitating the comparison between motor unit and SEMG frequency content.

In addition to the analyses described above, we studied the relationship between motor unit firing rate modulation and respiratory rate. Via frequency analysis of the motor unit data, we identified a rhythmic component of firing rate modulation at the frequency of the respiratory rate (whose frequency content we analyzed in a way similar to the one described above for the motor unit and RMS-SEMG data).

Thus, we investigated the phase relationship between respiratory rate and the rhythmic component of motor unit firing rate modulation observed at a frequency corresponding to the respiratory rate. Because of the non-stationarity of the motor unit firing rate time series, we decide to use a method previously developed by our team (Bonato et al. 2003) that relies on Cohen Class time-frequency transformations (Cohen 1995) to estimate phase relationships for time-variant signals. The technique leverages on a property of cross-time-frequency representations of the Cohen Class, namely that the ratio between imaginary and real part of a cross-transformation for a given frequency value provides an estimate of the phase relationship between the two signals under consideration (Bonato et al. 2000). Given the non-stationarity of the time series, this estimation technique allows one to select the intervals of stationarity on the cross-time-frequency representation where it is possible to identify intervals of coherent oscillation of the motor unit firing rate time series and the respiratory signal (Bonato et al. 2003). In this study, estimates of the phase ratio between the rhythmic component of modulation of the monitored motor unit and the recorded respiratory rate were derived by averaging the results of the referenced technique within intervals of coherent oscillation. Histograms of the estimated phase ratios were then built by analyzing multiple recordings to explore dominant behaviors of motor units in relation to respiratory phenomena.

RESULTS

Respiratory modulation of firing rates, ranging from 2 to 5 pps peak-to-peak for the continuous firing motor units, was observed for all of 32 trains of motor unit firings from 12 experiments with low-level, constant-amplitude contractions (amplitude range 0.9 - 2.4% EMG_{max}). Mean firing rates were close to 10 pulses per second (pps; Westad et al. 2004).

Respiratory modulation of firing rate is illustrated in Figure 1 for a 2-min contraction at constant, low amplitude (1.7% EMG_{max}; RMS-detected SEMG signal in top right panel). A motor unit, firing near threshold for recruitment, showed prominent modulation of firing at the respiratory frequency (Figure 1, top left). In the second row of Figure 1, the magnitude square of the Fourier transform of the motor unit train (left) and the SEMG signal (right) are displayed for wide (0-4 Hz) and low (0-1 Hz; inset) frequency ranges. The frequency content of the respiratory chest movement, at about 0.4 Hz, is shown by dashed line. The lower panels show the time-frequency representation of the motor unit firing rate (left) and the SEMG signal (right) estimated as explained in the Methods section. The respiratory frequency is prominently and consistently present in the time-frequency representation of the firing rate time-series. A small peak at twice the respiratory frequency is observed. There are indications of oscillatory activity at respiratory frequency in sections of the SEMG recording, but are much reduced relative to firing rate modulation of the recorded motor unit. The peak at respiratory frequency does not stand out from peaks at other frequencies in the SEMG frequency content (second row, right).

Contractions of 10 min duration were carried out with amplitudes ranging from 2.4 to 5.9% EMG_{max}. At low amplitudes, firing rates with 2-5 pps modulation at the respiratory frequency were observed, similar to the 2-min low-amplitude recordings. Firing rate modulation was markedly reduced in higher amplitude contractions (>3% EMG_{max}) and was in most recordings only detected through frequency analysis. However, three of 36 motor unit recordings showed strong respiratory modulation, similar to that observed in low-amplitude contractions. These recordings were not distinguished with respect to contraction level (ranging from 3.7 to 5.2% EMG_{max}) or recruitment threshold.

Figure 2 illustrates the effect of reduced respiratory firing rate modulation with increasing contraction amplitude. Constant-amplitude contractions of 10 min duration at 2.4% (A), 3.7% (B) and 5.5% (C) EMG_{max} are shown. Three to five motor units were recorded in each contraction. The plots in left column show frequency content of motor unit firing rates (note different y-calibration in Figure 2A); the plots in right column show frequency content of the SEMG amplitude modulation. Insets in Figure 2A and 2B show representative sections of firing rate modulation for the motor units with highest and lowest peak at respiratory frequency in the respective recordings. The plots are similarly calibrated in arbitrary units (a.u.) to allow comparison of plots from different panels. The highest peak (0.8 a.u., Figure 2A) represents a modulation of 5 pps with mean firing rate 10.1 pps. Peaks of 0.2 a.u. correspond to firing rate modulation of ~2 pps and are detectable by visual inspection of the recording. Peaks <0.1 a.u. in the frequency plots are usually not detected by visual inspection of the recording. Respiratory modulation of firing rates in the contraction at 5.5% EMG_{max} was barely detected in the frequency plots (Figure 2C). Respiratory modulation was much attenuated or absent in the SEMG signal. A weak peak at respiratory frequency, typical of the SEMG signal in low-amplitude contractions, is seen in the top right panel (see also Figure 1).

A small peak at twice the respiratory frequency is noted in the frequency plots of firing rate modulation for the recording shown in Figure 2A. Analysis by time-frequency representation showed a dynamic interaction of the respiratory-related frequency components, with modulation at the first harmonic frequency dominant about 50 s into the recording (Figure 3). Later in the contraction this frequency component disappeared. The same dynamic development of frequency content was found for the other recorded motor units in this experiment.

Harmonic components in frequency representations may arise from non-sinusoidal modulation at the fundamental frequency. Three experiments with synchronized recordings of EMG and respiratory movement were therefore examined to clarify the nature of the respiratory modulation. Two 20-s sections showing chest movement and firing rates of three motor units were selected from each of two recordings (Figure 4). Firing rate modulation at double the respiratory frequency is observed in the top left panel, changing to dominant modulation at the respiratory frequency (top right). In Figure 4B firing rates were modulated at the respiratory frequency in most cycles, but with a tendency of a harmonic frequency component in the first and third breaths (left panel). The bottom motor unit, which is firing near threshold and has the strongest firing rate modulation, was phase-shifted relative to the other two motor units in most breaths, but has similar respiratory phase to the other two motor units in a few breaths (e.g., last breath in left panel). Firing rate modulation at higher frequencies (1-2 Hz) was synchronized throughout the recordings, consistent with the “common drive” hypothesis of command signals from supraspinal centers to the motor nuclei (De Luca et al. 1982; De Luca and Erim 1994).

Figure 5 shows phase of respiratory modulation of firing rates relative to chest movement, based on motor unit recordings from three experiments with constant-amplitude contractions. Most motor units had peak firing rates at the transition from inspiration to expiration. A smaller group presented peak firing rates at the transition from expiration to inspiration. A few motor units were firing in phase with peak inspiratory movement and none were firing in phase with expiration.

The effect of task condition was examined by comparing the frequency content of motor unit firing patterns in typing and mental stress experiments with frequency spectra obtained in constant amplitude contractions. Some experiments allowed reliable tracking of the same motor units in different tasks. Respiratory modulation of firing rates was much reduced in the experiments with mental stress and typing. The SEMG amplitude was usually low in the experiments with mental stress (mean 1.6%, range 0.5-2.6% EMG_{max}). Eleven motor units with stable firing were recorded. Evidence of low respiratory modulation was observed in some of the firing patterns, contrasting the consistent and prominent firing rate modulation in constant-amplitude contractions of similar low amplitude.

Mean SEMG amplitude in typing without stress varied considerably (group mean 4.1%, range 1.3 to 9.1% EMG_{max}). Respiratory modulation of firing rates was rarely observed, even for contractions with very low SEMG amplitude: only three of 50 motor units in typing without imposed stress showed unequivocal evidence of respiratory modulation. In typing with stress 11 motor units were analyzed and only one showed evidence of respiratory modulation. However, the firing pattern of motor units in typing tasks was quite variable at low contraction amplitudes, presumably adjusting to variation in force demand due to arm movement, which may mask any respiratory firing rate modulation.

Frequency plots of firing rate and SEMG modulation are shown in Figure 6 for two experiments with mental stress where motor units had stable firing patterns. The recordings in Figure 6A and B are from the same experiments as shown in Figure 2A and B, and with the same motor units. The power density spectra of motor unit firing rate modulation in Figure 6 (left panels) show no clear indication of respiratory rhythm in the mental stress experiments, despite SEMG amplitudes lower than the corresponding constant amplitude contractions (Figure 2A, B). Time plots of heart rate, SEMG and motor unit firing rates in the mental stress experiment of Figure 6B are shown in Figure 7. This subject adopted a nominally relaxed, seated posture. She received information about the task and the opportunity of a money reward two min before the start of the task, responding with 10 beats-per-min elevation of the heart rate. The motor units were already firing when the experiment started and maintained stable firing during the experiment, except for a silent period for two of the motor units. The silent periods coincided with temporary depressions in heart rate.

DISCUSSION

Trapezius motoneurons show respiratory firing rate modulation in low-level, constant amplitude contractions. The phase relative to chest movement is variable. Most motor units have peak firing rate in the transition from inspiration to expiration, but peak firing in the transition from expiration to inspiration is also common. SEMG activity, indicating force development in upper trapezius, shows little or no respiratory modulation. Respiratory firing rate modulation is markedly reduced when low-level motor activity is elicited in situations with mental stress.

Trapezius is frequently included as an accessory respiratory muscle with inspiratory phasing in the literature dealing with respiratory motor control (Campbell 1968; Zemlin 1997). Respiratory modulation of trapezius SEMG activity with inspiratory phasing has been reported for “chest breathers” (Schleifer et al. 2002). Other scientists have failed to find inspiratory modulated trapezius muscle activity and consider the muscle not to have a respiratory function (Basmajian and De Luca 1985). Conversely, expiratory phased activity pattern is observed for subjects performing forceful phonation as in operatic singing (Pettersen and Westgaard 2004).

The expiratory phased activity pattern is reduced or disappears when the breathing demand in phonation is lowered (Pettersen et al. 2004). Thus, trapezius may contribute in a respiratory capacity in different ways, but does not normally contribute to respiratory chest movement in quiet breathing. This is the relevant breathing requirement for subjects performing the light motor tasks of this study and is consistent with the lack of respiratory modulation in the SEMG signal. The respiratory firing rate modulation in low-level, constant amplitude contractions was therefore a surprise. Inconsistent phase association of motor unit firing patterns with respiratory movement and relatively large number of motor units active at low SEMG amplitude (Westad and Westgaard 2005) may explain the lack of respiratory modulation in the SEMG signal.

Respiratory modulated trapezius motor unit firing pattern, with peak firing rates in the transition between inspiration and expiration or *vice versa* and periods with modulation at double the respiratory frequency suggest that the observed respiratory modulation is not generated in response to a task-specific central command signal. Both respiratory phases can be simultaneously present in the firing pattern, usually with one phase dominant. Sections of recordings with a dominant first harmonic frequency component may be a special case with balanced excitation from two independent, respiratory-modulated inputs with opposite respiratory phase.

The source of respiratory modulated excitatory input to trapezius motoneurons is unknown. One possibility is peripheral afferent input from diverse populations of mechanoreceptors, sensitive to respiratory movement of either phase. However, respiratory modulation is reported for lumbar motoneurons in cats under strong respiratory drive through breathing O_2-enriched air with added CO_2 (Kirkwood et al. 2002). The cats were paralyzed and artificially ventilated, i.e., without peripheral afferent input to motoneurons. The central respiratory drive potential (CRDP) was usually locked to early expiration, but also showed variation in phase, analogous to the variation in respiratory phase of motor unit firing patterns in this study (cf. Figure 4). Another study reported CRDPs in a (small) fraction of lumbar motoneurons in decerebrate, unanesthesized cats (Meyer-Lohmann 1974). Respiratory modulated firing of triceps surae spindle afferents was observed; however, this modulation disappeared when the fusimotor innervation was disrupted, indicating that the afferent respiratory modulation was due to gamma motor activity.

Another consideration, pointing to a possible source of respiratory excitation, is based on developmental similarities of trapezius motoneurons to motoneurons of other brain stem and medullary motor nuclei. Trapezius motoneurons are located in the spinal accessory (XI) nucleus and derive from the group of special visceral neurons (Kandel et al. 1991). Motoneurons of this origin (from the trigeminal, facial, vagus motor and spinal accessory nuclei) control muscles involved in vegetative function and cognitive-behavioral expression, including control of facial expression, mastication, speech and upper airway resistance. Several of these motor nuclei, and also the hypoglossal nuclei (controlling muscles of the tongue) include motoneurons with respiratory modulated activity of variable phase, typically with peak firing in the transition from inspiration to expiration or *vice versa* (Huangfu et al. 1993; Merrill 1970; Peever et al. 2001). Hypoglossal motoneurons have been studied in some detail, with a view to determining the location of respiratory rhythm generation (Duffin 2004). Neurons with peak firing in the transition from inspiration to expiration (post-inspiratory or expiratory decrementing) are considered to control airway muscle activity. Hypoglossal motoneurons and interneurons within the hypoglossal motor nuclei receive early inspiratory input from a source tentatively located to the lateral tegmental field, separate from inspiratory neurons of the rostral ventral respiratory group (Peever et al. 2002). The inspiratory drive is to a considerable extent mediated through interneurons, similar to the respiratory drive to thoracic respiratory motoneurons (Kirkwood et al. 1993). Active inhibition of hypoglossal motoneurons during expiration was not detected, indicating that repolarization was due to disfacilitation, as was also the case for early inspiratory facial motoneurons (Huangfu et al. 1993). Conceivably, trapezius motoneurons may have a similar organization of their pre-motor input.

The different respiratory modes of trapezius, determined by respiratory demand and habitual differences in breathing pattern, highlight the complex respiratory function of trapezius. The putative CRDPs must be weak in normal breathing, presumably explaining the observation of respiratory firing modulation only in low-amplitude contractions. Trapezius has an integrative role in postural control; it contributes in head movement and to positioning and stabilization of the shoulder in arm movement. This further argues for active inhibition locked to respiratory phase is less prominent in trapezius than for primary respiratory muscles such as the diaphragm and the intercostals (Aminoff and Sears 1971).

CRDPs in lumbar motoneurons seem to be promoted by motoneuron plateau potentials (Kirkwood et al. 2002). Trapezius motoneuron firing patterns show several features indicative of plateau potentials (Bennett et al. 1998; Hornby et al. 2002), including derecruitment at a lower central drive than at recruitment (Westad et al. 2003) and the “warm-up” phenomenon (Westad et al. 2004), all observed in constant-amplitude contractions, indicating that plateau potentials are generally present in active trapezius motoneurons.

Respiratory modulation of trapezius motor units is much reduced in mental stress experiments with low contraction amplitude. The mental stress procedure induces autonomic (elevated heart and respiratory rate, increased blood pressure and decreased skin blood flow, Nilsen et al. submitted) and muscular responses (Bansevicius et al. 1997), the latter prominent for the frontalis and trapezius muscles (Wærsted and Westgaard 1996). Plateau potentials in motoneurons are promoted by monoaminergic pathways from the brain stem (Kiehn et al. 1996; Kiehn and Eken 1998). These facilitate motor activity and activate the sympatho-adrenal system, thereby producing an autonomic arousal response (e.g., Jacobs et al. 1991; Jacobs and Fornal 1999). The serotonergic system is not responsive to stress-type challenges (Jacobs et al. 2002) while the noradrenergic locus coeruleus neurons show immediate elevated firing in such situations (Aston-Jones and Bloom 1981; Jacobs 1986; Carrasco and Van de Kar 2003). The mechanism for control of motoneuron excitability is unknown. It may involve control of motoneuron plateau potentials, which are likely activated also in constant-amplitude contractions (Westad et al. 2003; Westad et al. 2004). Noradrenergic input to the spinal cord also influence excitation of segmental interneurons, facilitating monosynaptic peripheral afferent reflexes (Chan et al. 1986) and suppressing the Renshaw system of interneurons (Fung et al. 1987).

The consistent and prominent respiratory modulation of firing in constant-amplitude contractions indicates that the respiratory premotor input and a supraspinal command signal to elevate the shoulders act in synergy to activate segmental interneurons and motoneurons. The marked attenuation of respiratory modulation in the mental stress experiments indicates a competitive interaction, possibly representing a state change in the segmental spinal cord inter-neuronal network

(Burke 1999). Spinal network with different excitatory effects on motor nuclei, coinciding with distinct changes in animal behavior, is demonstrated for the cervical spinal cord of cats (e.g., Alstermark et al. 1987; Lundberg 1999) and probably exist in the human (Pierrot-Deseilligny 1996). Task-dependent differences in firing rate modulation may provide clues regarding premotor pathways providing excitatory input to trapezius motoneurons.

Few examples of respiratory modulation of firing rates was observed in the typing experiments, but contraction amplitude was relatively high in experiments with stable motoneuron firing, while intermittent firing patterns were common in low-amplitude contractions. Respiratory modulation of firing rates may thus exist in the typing experiments, but be masked. It may alternatively be argued the circumstances for trapezius motor activity in typing, supporting shoulder posture in arm movement, is sufficiently different to constant amplitude contractions to warrant a different state of spinal cord networks. Further experimentation, e.g., controlled arm movement, may allow further examination of this point.

Ongoing excitatory input to trapezius motoneurons from a variety of sources, including respiratory modulated input of either phase, may contribute to the preponderance of “spontaneous” low-level trapezius motor activity. Such activity is observed both in laboratory (Bansevicius et al. 1997) and vocational (Vasseljen and Westgaard 1995) settings.

In conclusion, we argue that respiratory modulation of low-threshold trapezius motoneurons in constant amplitude contractions most likely is due to respiratory modulated central input. The reduction in respiratory modulation of firing rate in experiments with imposed mental stress may be due to a task-dependent change in local interneuronal networks, triggered by an arousal response mediated through monoaminergic influences on the motor system.

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FIGURE LEGENDS

Figure 1. Frequency content of motor unit firing pattern (left) and surface electromyographic (SEMG) activity (right) in a 2-min constant amplitude contraction at 1.7% EMG_{max}. The top row shows time plots of motor unit firing rate with respiratory modulation (left) and simultaneously recorded SEMG activity (right). Firing rate modulation at the respiratory frequency was confirmed in similar analyzed, separate recording of chest movement. Second row shows frequency plots (magnitude square of the Fourier transform) of firing rate (left) and SEMG activity (right). The frequency content is shown at two resolutions, 0-1 Hz (insets) and 0-4 Hz. In left panel frequency content of respiratory chest movement is shown by dashed line. Bottom row shows waterfall plots depicting time-frequency representations of frequency content in motor unit firing rate (left) and SEMG signal (right). The frequency spectrum is calibrated in arbitrary units (a.u.), but the same calibration procedure is used in all frequency analyses presented in this paper. MU: motor unit; PSD: power spectral density

Figure 2. Frequency content of motor unit firing patterns (left) and the simultaneously recorded SEMG activity pattern (right) of three constant amplitude contractions performed at increasing amplitude from A to C (contraction amplitudes indicated in right panels). Three to five motor units were recorded in each contraction. Insets in A, B show representative time plots of sections of recording for the motor units with the highest and lowest respiratory modulation in C. Respiratory modulation of firing rates was not detected in the last experiment. Vertical calibration bar: 5 pulses per second (pps); horizontal bar 5 s.

Figure 3. Waterfall plot showing time-frequency representation of motor unit firing pattern. One of the motor units with averaged frequency content shown in Figure 2A was selected. The other motor units in the same experiment show similar time development of frequency content in their firing pattern.

Figure 4. Time plots of firing rate modulation for three motor units (top three traces) and upper chest movement (bottom trace). Two panels from each of two constant-amplitude experiments are shown, selected to illustrate specific features in the firing pattern. Firing rates are low-pass filtered with cut-off frequency 4 and 1 Hz, respectively. Firing patterns with the higher filter setting are shown as thin-line traces superimposed on the thicker lines, representing firing rates with the lower filter setting.

Figure 5. Histogram showing respiratory phase (i.e., highest firing rate) of 46 motor units in 3 constant amplitude experiments with synchronized recordings of motor units and chest movement.

Figure 6. Frequency content of motor unit firing patterns (left) and simultaneously recorded SEMG activity pattern (right) of two contractions with mental stress (A, B). Recordings were from the same experiment as illustrated in Figure 2A, B. Three (A) and four (B) motor units were recorded. Contraction amplitudes are indicated in panels on right.

Figure 7. Time plots of heart rate, root-mean-square detected SEMG signal and firing rates of four motor units (marked 1 to 4), recorded in the experiment with mental stress shown in Figure 6B. Heart rate was continuously recorded and time intervals before (negative time indication) and after the cessation of the experiment is shown for this variable. Vertical arrows in top panel mark start and end of the experiment. Motor unit templates at the beginning and end of the experiment (motor units 1 to 3) and the start and end of a period without firing for motor unit 4, marked by asterisks, are shown next to the plots of firing rates. Horizontal bars in the two top panels indicate time intervals with no firing for motor units 2 and 4. Note temporary reductions in heart rate coinciding with the silent periods of motor unit firing.

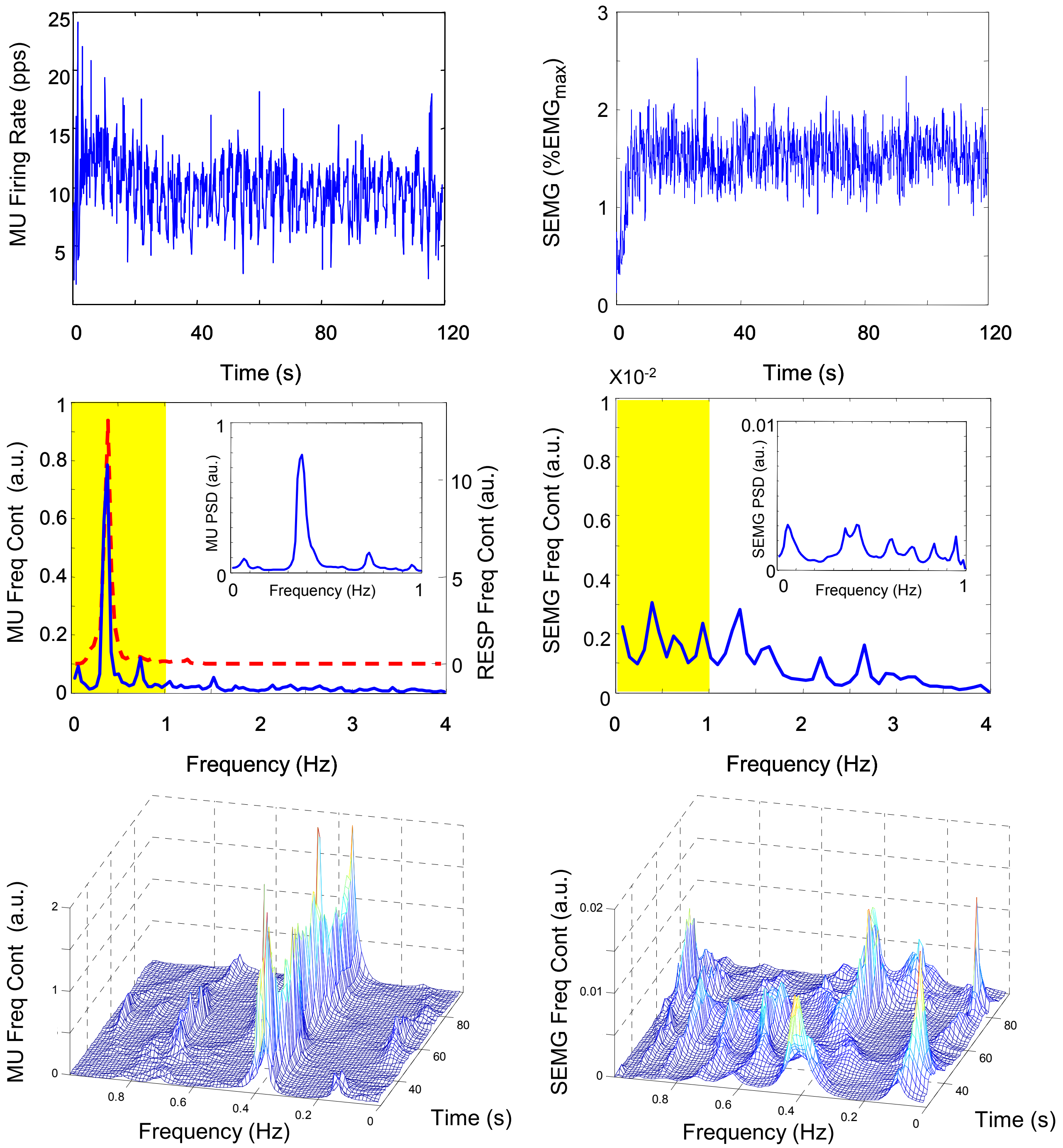

MU Firing Rate (pps)
25
20
15
10
5
0
40
80
120
Time (s)
SEMG (%EMG$_{max}$)
3
2
1
0
0
40
80
120
Time (s)
MU Freq Cont (a.u.)
1
0.8
0.6
0.4
0.2
0
MU PSD (au.)
1
0
0
Frequency (Hz)
1
10
5
0
RESP Freq Cont (a.u.)
0
1
2
3
4
Frequency (Hz)
X10^{-2}
SEMG Freq Cont (a.u.)
1
0.8
0.6
0.4
0.2
0
0.01
SEMG PSD (au.)
0
0
Frequency (Hz)
1
0
1
2
3
4
Frequency (Hz)
MU Freq Cont (a.u.)
2
1
0
0.8
0.6
0.4
0.2
0
Frequency (Hz)
40
60
80
Time (s)
SEMG Freq Cont (a.u.)
0.02
0.01
0
0.8
0.6
0.4
0.2
0
Frequency (Hz)
40
60
80
Time (s)

Figure 1

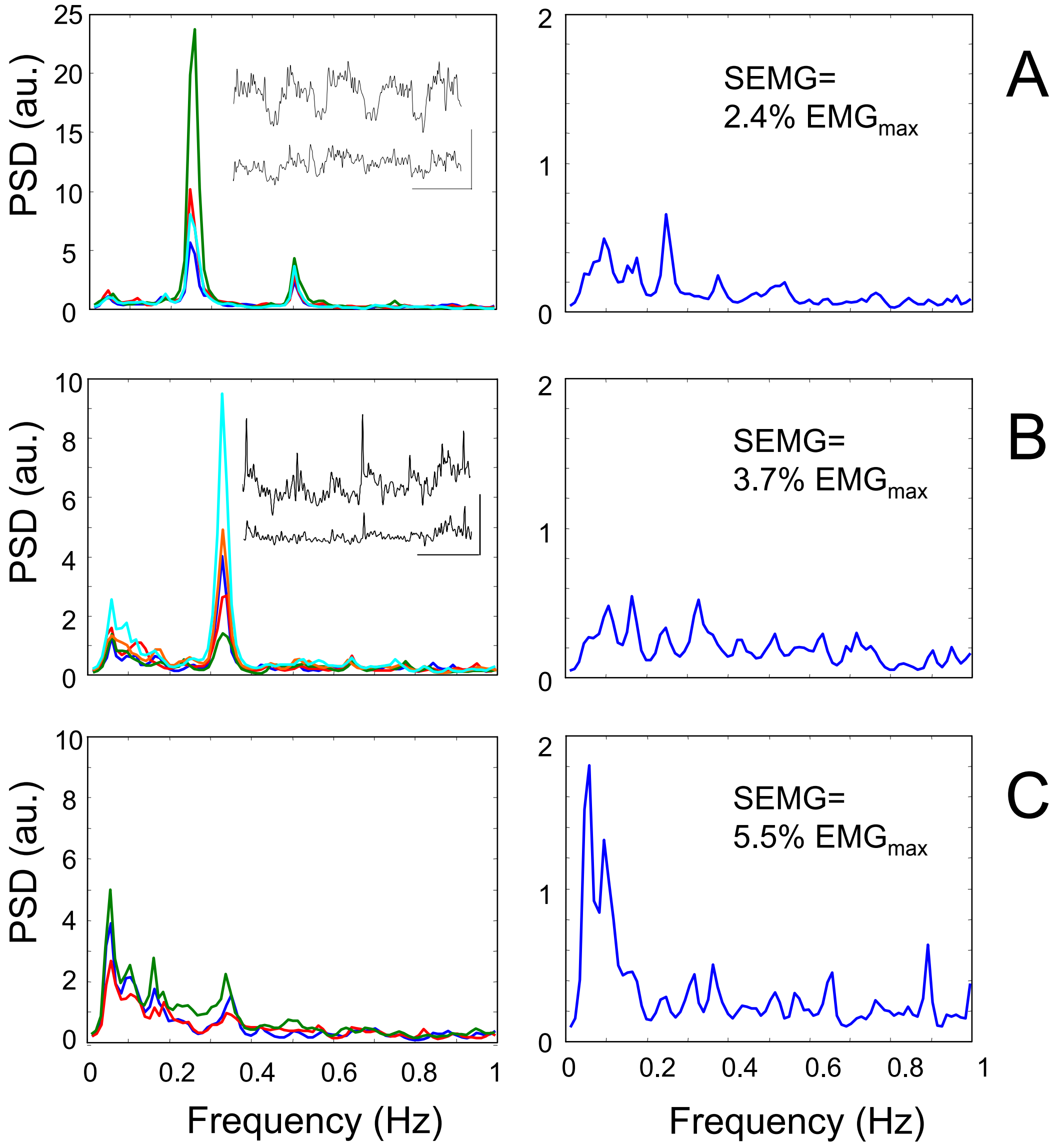

PSD (au.)
25
20
15
10
5
0
2
1
0
SEMG=
2.4% EMG$_{max}$
A
10
8
6
4
2
0
SEMG=
3.7% EMG$_{max}$
B
SEMG=
5.5% EMG$_{max}$
C
0
0.2
0.4
0.6
0.8
1
Frequency (Hz)

Figure 2

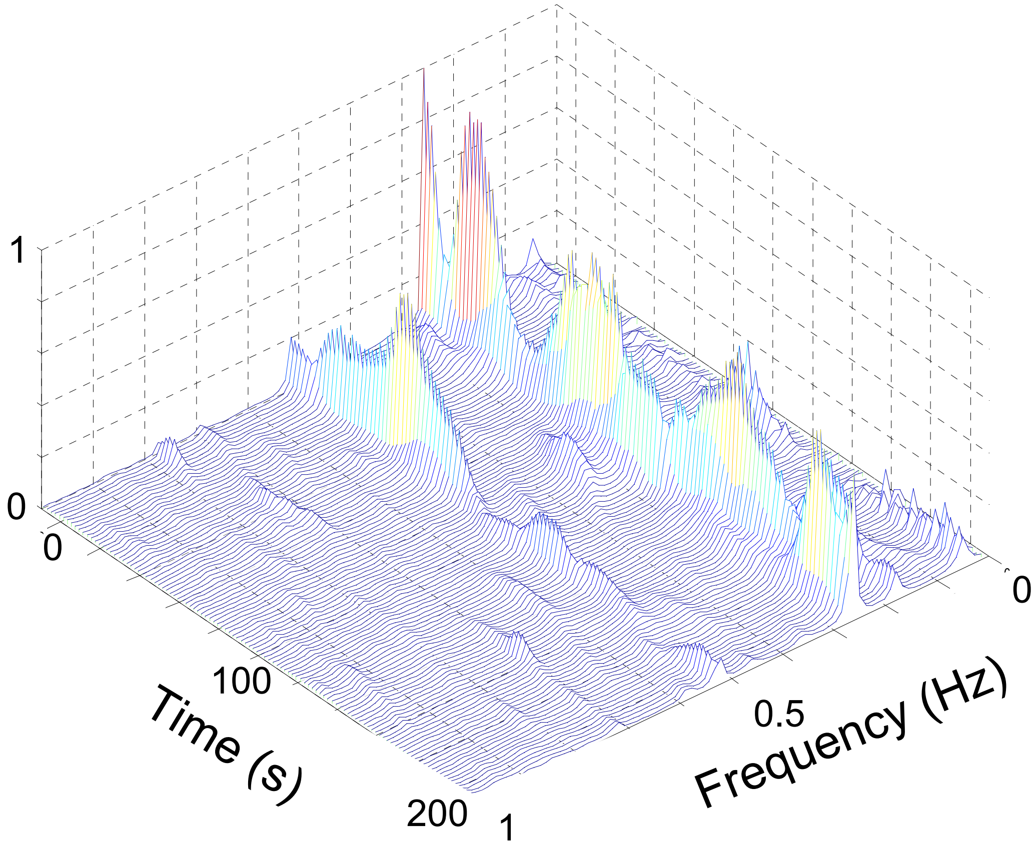


Figure 3

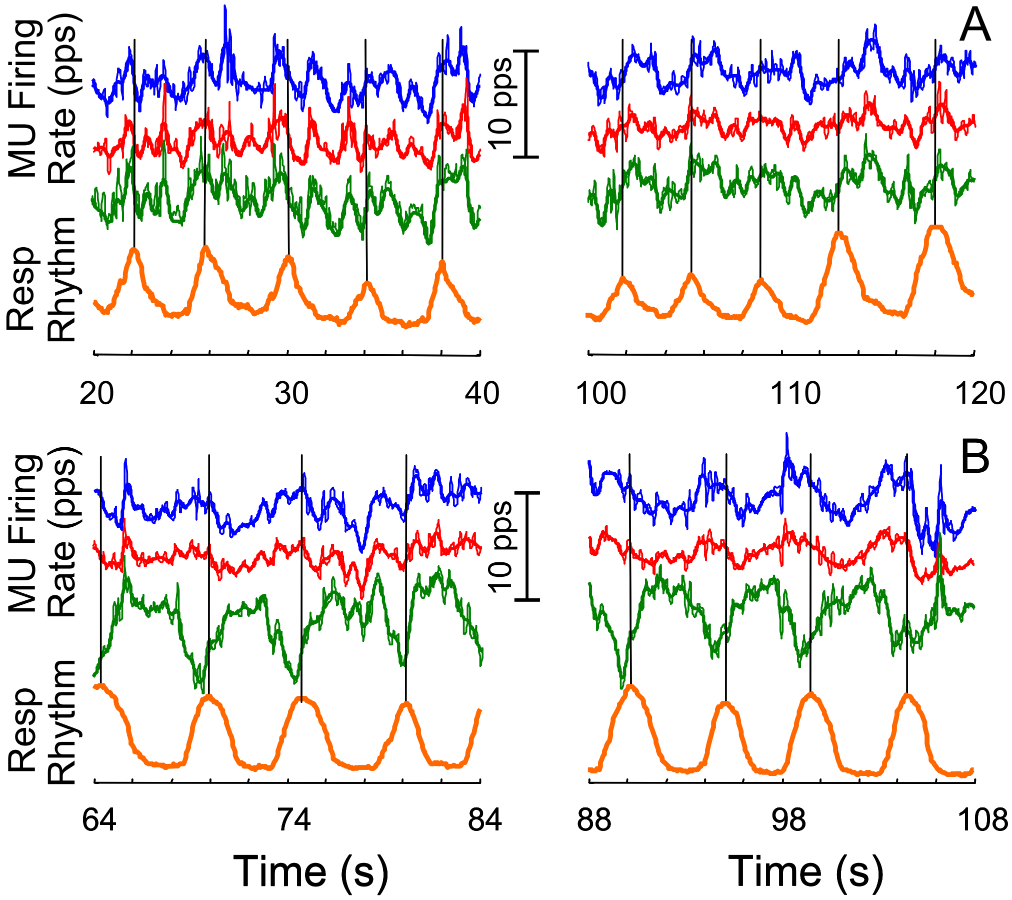

A
MU Firing Rate (pps)
Resp Rhythm
10 pps
20
30
40
100
110
120
B
MU Firing Rate (pps)
Resp Rhythm
10 pps
64
74
84
88
98
108
Time (s)
Time (s)

Figure 4

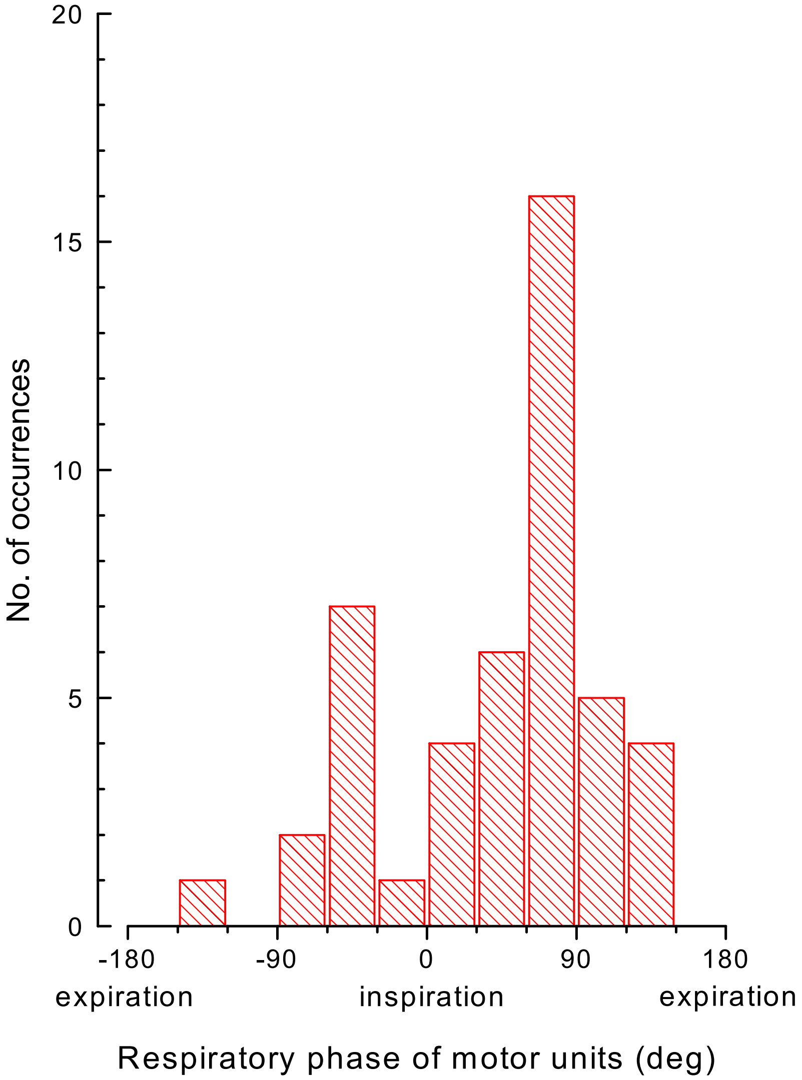
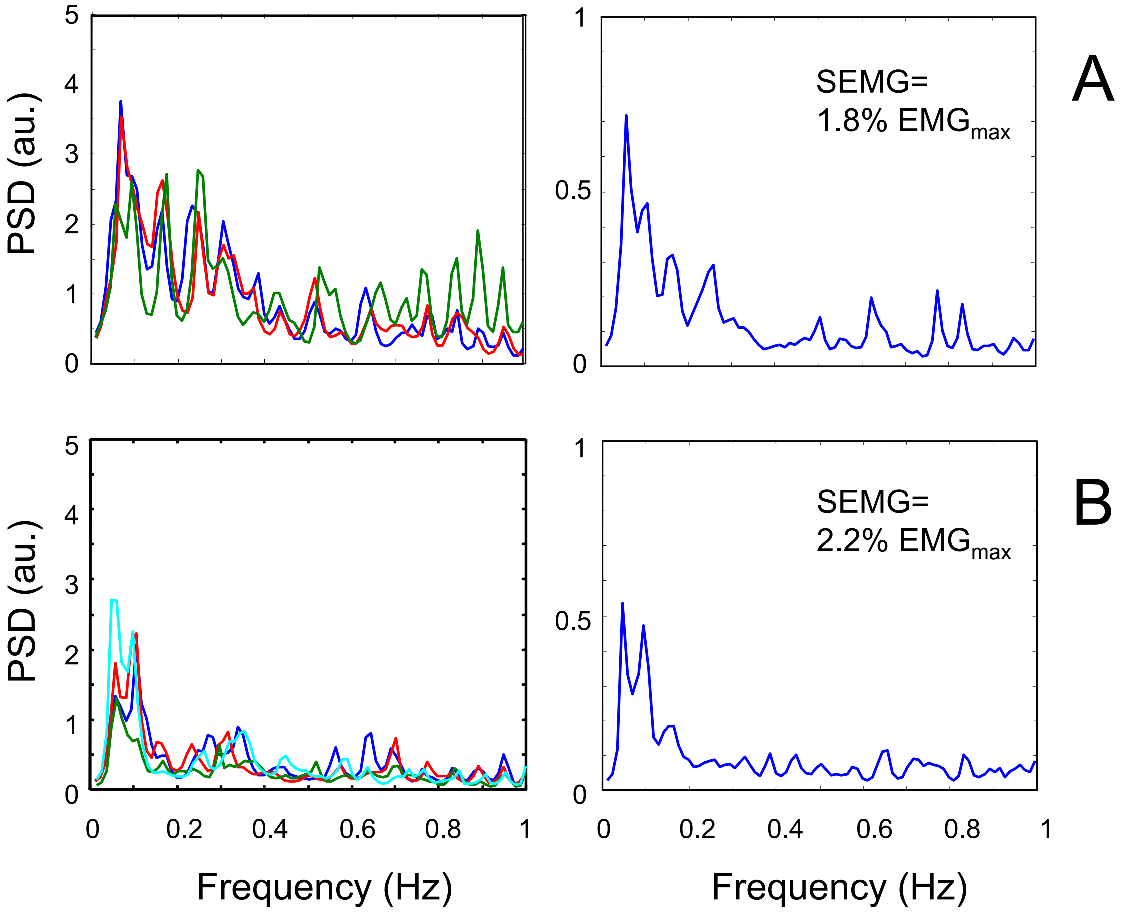
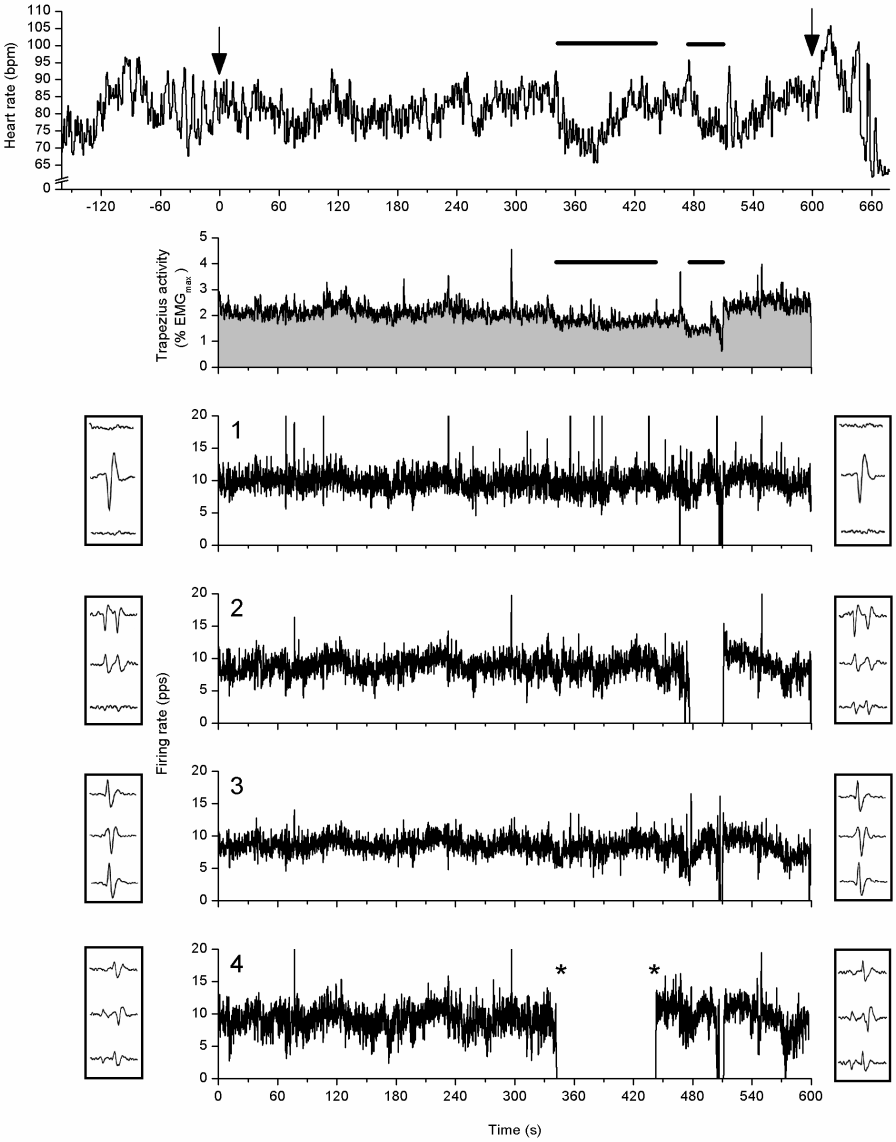

20
15
10
5
0
No. of occurrences
-180
-90
0
90
180
expiration
inspiration
expiration
Respiratory phase of motor units (deg)

Figure 5



PSD (au.)
5
4
3
2
1
0
1
0.5
0
SEMG=
1.8% EMG$_{max}$
A
PSD (au.)
5
4
3
2
1
0
1
0.5
0
SEMG=
2.2% EMG$_{max}$
B
0
0.2
0.4
0.6
0.8
1
Frequency (Hz)
0
0.2
0.4
0.6
0.8
1
Frequency (Hz)

Figure 6



Heart rate (bpm)
110
105
100
95
90
85
80
75
70
65
0
-120
-60
0
60
120
180
240
300
360
420
480
540
600
660
Trapezius activity (% EMG$_{max}$)
5
4
3
2
1
0
Firing rate (pps)
20
15
10
5
0
1
2
3
4
*
*
Time (s)

Figure 7

Doctoral theses in Biology
Norwegian University of Science and Technology

Year	Name	Degree	Title
1974	Tor-Henning Iversen	Dr. philos Botany	The roles of statholiths, auxin transport, and auxin metabolism in root gravitropism
1978	Tore Slagsvold	Dr. philos. Zoology	Breeding events of birds in relation to spring temperature and environmental phenology.
1978	Egil Sakshaug	Dr.philos Botany	"The influence of environmental factors on the chemical composition of cultivated and natural populations of marine phytoplankton"
1980	Arnfinn Langeland	Dr. philos. Zoology	Interaction between fish and zooplankton populations and their effects on the material utilization in a freshwater lake.
1980	Helge Reinertsen	Dr. philos Botany	The effect of lake fertilization on the dynamics and stability of a limnetic ecosystem with special reference to the phytoplankton
1982	Gunn Mari Olsen	Dr. scient Botany	Gravitropism in roots of *Pisum sativum* and *Arabidopsis thaliana*
1982	Dag Dolmen	Dr. philos. Zoology	Life aspects of two sympartic species of newts (*Triturus, Amphibia*) in Norway, with special emphasis on their ecological niche segregation.
1984	Eivin Røskaft	Dr. philos. Zoology	Sociobiological studies of the rook *Corvus frugilegus.*
1984	Anne Margrethe Cameron	Dr. scient Botany	Effects of alcohol inhalation on levels of circulating testosterone, follicle stimulating hormone and luteinzing hormone in male mature rats
1984	Asbjørn Magne Nilsen	Dr. scient Botany	Alveolar macrophages from expectorates – Biological monitoring of workers exosed to occupational air pollution. An evaluation of the AM-test
1985	Jarle Mork	Dr. philos. Zoology	Biochemical genetic studies in fish.
1985	John Solem	Dr. philos. Zoology	Taxonomy, distribution and ecology of caddisflies (*Trichoptera*) in the Dovrefjell mountains.

1985	Randi E. Reinertsen	Dr. philos. Zoology	Energy strategies in the cold: Metabolic and thermoregulatory adaptations in small northern birds.
1986	Bernt-Erik Sæther	Dr. philos. Zoology	Ecological and evolutionary basis for variation in reproductive traits of some vertebrates: A comparative approach.
1986	Torleif Holthe	Dr. philos. Zoology	Evolution, systematics, nomenclature, and zoogeography in the polychaete orders *Oweniimorpha* and *Terebellomorpha*, with special reference to the Arctic and Scandinavian fauna.
1987	Helene Lampe	Dr. scient. Zoology	The function of bird song in mate attraction and territorial defence, and the importance of song repertoires.
1987	Olav Hogstad	Dr. philos. Zoology	Winter survival strategies of the Willow tit *Parus montanus*.
1987	Jarle Inge Holten	Dr. philos Bothany	Autecological investigations along a coust-inland transect at Nord-Møre, Central Norway
1987	Rita Kumar	Dr. scient Botany	Somaclonal variation in plants regenerated from cell cultures of *Nicotiana sanderae* and *Chrysanthemum morifolium*
1987	Bjørn Åge Tømmerås	Dr. scient. Zoology	Olfaction in bark beetle communities: Interspecific interactions in regulation of colonization density, predator - prey relationship and host attraction.
1988	Hans Christian Pedersen	Dr. philos. Zoology	Reproductive behaviour in willow ptarmigan with special emphasis on territoriality and parental care.
1988	Tor G. Heggberget	Dr. philos. Zoology	Reproduction in Atlantic Salmon (*Salmo salar*): Aspects of spawning, incubation, early life history and population structure.
1988	Marianne V. Nielsen	Dr. scient. Zoology	The effects of selected environmental factors on carbon allocation/growth of larval and juvenile mussels (*Mytilus edulis*).
1988	Ole Kristian Berg	Dr. scient. Zoology	The formation of landlocked Atlantic salmon (*Salmo salar* L.).

1989	John W. Jensen	Dr. philos. Zoology	Crustacean plankton and fish during the first decade of the manmade Nesjø reservoir, with special emphasis on the effects of gill nets and salmonid growth.
1989	Helga J. Vivås	Dr. scient. Zoology	Theoretical models of activity pattern and optimal foraging: Predictions for the Moose *Alces alces*.
1989	Reidar Andersen	Dr. scient. Zoology	Interactions between a generalist herbivore, the moose *Alces alces*, and its winter food resources: a study of behavioural variation.
1989	Kurt Ingar Draget	Dr. scient Botany	Alginate gel media for plant tissue culture,
1990	Bengt Finstad	Dr. scient. Zoology	Osmotic and ionic regulation in Atlantic salmon, rainbow trout and Arctic charr: Effect of temperature, salinity and season.
1990	Hege Johannesen	Dr. scient. Zoology	Respiration and temperature regulation in birds with special emphasis on the oxygen extraction by the lung.
1990	Åse Krøkje	Dr. scient Botany	The mutagenic load from air pollution at two work-places with PAH-exposure measured with Ames Salmonella/microsome test
1990	Arne Johan Jensen	Dr. philos. Zoology	Effects of water temperature on early life history, juvenile growth and prespawning migrations of Atlantic salmion (*Salmo salar*) and brown trout (*Salmo trutta*): A summary of studies in Norwegian streams.
1990	Tor Jørgen Almaas	Dr. scient. Zoology	Pheromone reception in moths: Response characteristics of olfactory receptor neurons to intra- and interspecific chemical cues.
1990	Magne Husby	Dr. scient. Zoology	Breeding strategies in birds: Experiments with the Magpie *Pica pica*.
1991	Tor Kvam	Dr. scient. Zoology	Population biology of the European lynx (*Lynx lynx*) in Norway.
1991	Jan Henning L'Abêe Lund	Dr. philos. Zoology	Reproductive biology in freshwater fish, brown trout *Salmo trutta* and roach *Rutilus rutilus* in particular.

1991	Asbjørn Moen	Dr. philos Botany	The plant cover of the boreal uplands of Central Norway. I. Vegetation ecology of Sølendet nature reserve; haymaking fens and birch woodlands
1991	Else Marie Løbersli	Dr. scient Botany	Soil acidification and metal uptake in plants
1991	Trond Nordtug	Dr. scient. Zoology	Reflctometric studies of photomechanical adaptation in superposition eyes of arthropods.
1991	Thyra Solem	Dr. scient Botany	Age, origin and development of blanket mires in Central Norway
1991	Odd Terje Sandlund	Dr. philos. Zoology	The dynamics of habitat use in the salmonid genera *Coregonus* and *Salvelinus*: Ontogenic niche shifts and polymorphism.
1991	Nina Jonsson	Dr. philos.	Aspects of migration and spawning in salmonids.
1991	Atle Bones	Dr. scient Botany	Compartmentation and molecular properties of thioglucoside glucohydrolase (myrosinase)
1992	Torgrim Breiehagen	Dr. scient. Zoology	Mating behaviour and evolutionary aspects of the breeding system of two bird species: the Temminck's stint and the Pied flycatcher.
1992	Anne Kjersti Bakken	Dr. scient Botany	The influence of photoperiod on nitrate assimilation and nitrogen status in timothy (*Phleum pratense* L.)
1992	Tycho Anker-Nilssen	Dr. scient. Zoology	Food supply as a determinant of reproduction and population development in Norwegian Puffins *Fratercula arctica*
1992	Bjørn Munro Jenssen	Dr. philos. Zoology	Thermoregulation in aquatic birds in air and water: With special emphasis on the effects of crude oil, chemically treated oil and cleaning on the thermal balance of ducks.
1992	Arne Vollan Aarset	Dr. philos. Zoology	The ecophysiology of under-ice fauna: Osmotic regulation, low temperature tolerance and metabolism in polar crustaceans.
1993	Geir Slupphaug	Dr. scient Botany	Regulation and expression of uracil-DNA glycosylase and O^6-methylguanine-DNA methyltransferase in mammalian cells
1993	Tor Fredrik Næsje	Dr. scient. Zoology	Habitat shifts in coregonids.

1993	Yngvar Asbjørn Olsen	Dr. scient. Zoology	Cortisol dynamics in Atlantic salmon, *Salmo salar* L.: Basal and stressor-induced variations in plasma levels ans some secondary effects.
1993	Bård Pedersen	Dr. scient Botany	Theoretical studies of life history evolution in modular and clonal organisms
1993	Ole Petter Thangstad	Dr. scient Botany	Molecular studies of myrosinase in Brassicaceae
1993	Thrine L. M. Heggberget	Dr. scient. Zoology	Reproductive strategy and feeding ecology of the Eurasian otter *Lutra lutra*.
1993	Kjetil Bevanger	Dr. scient. Zoology	Avian interactions with utility structures, a biological approach.
1993	Kåre Haugan	Dr. scient Bothany	Mutations in the replication control gene trfA of the broad host-range plasmid RK2
1994	Peder Fiske	Dr. scient. Zoology	Sexual selection in the lekking great snipe (*Gallinago media*): Male mating success and female behaviour at the lek.
1994	Kjell Inge Reitan	Dr. scient Botany	Nutritional effects of algae in first-feeding of marine fish larvae
1994	Nils Røv	Dr. scient. Zoology	Breeding distribution, population status and regulation of breeding numbers in the northeast-Atlantic Great Cormorant *Phalacrocorax carbo carbo*.
1994	Annette-Susanne Hoepfner	Dr. scient Botany	Tissue culture techniques in propagation and breeding of Red Raspberry (*Rubus idaeus* L.)
1994	Inga Elise Bruteig	Dr. scient Bothany	Distribution, ecology and biomonitoring studies of epiphytic lichens on conifers
1994	Geir Johnsen	Dr. scient Botany	Light harvesting and utilization in marine phytoplankton: Species-specific and photoadaptive responses
1994	Morten Bakken	Dr. scient. Zoology	Infanticidal behaviour and reproductive performance in relation to competition capacity among farmed silver fox vixens, *Vulpes vulpes*.
1994	Arne Moksnes	Dr. philos. Zoology	Host adaptations towards brood parasitism by the Cockoo.
1994	Solveig Bakken	Dr. scient Bothany	Growth and nitrogen status in the moss *Dicranum majus* Sm. as influenced by nitrogen supply

1995	Olav Vadstein	Dr. philos Botany	The role of heterotrophic planktonic bacteria in the cycling of phosphorus in lakes: Phosphorus requirement, competitive ability and food web interactions.
1995	Hanne Christensen	Dr. scient. Zoology	Determinants of Otter *Lutra lutra* distribution in Norway: Effects of harvest, polychlorinated biphenyls (PCBs), human population density and competition with mink *Mustela vision*.
1995	Svein Håkon Lorentsen	Dr. scient. Zoology	Reproductive effort in the Antarctic Petrel *Thalassoica antarctica*; the effect of parental body size and condition.
1995	Chris Jørgen Jensen	Dr. scient. Zoology	The surface electromyographic (EMG) amplitude as an estimate of upper trapezius muscle activity
1995	Martha Kold Bakkevig	Dr. scient. Zoology	The impact of clothing textiles and construction in a clothing system on thermoregulatory responses, sweat accumulation and heat transport.
1995	Vidar Moen	Dr. scient. Zoology	Distribution patterns and adaptations to light in newly introduced populations of *Mysis relicta* and constraints on Cladoceran and Char populations.
1995	Hans Haavardsholm Blom	Dr. philos Bothany	A revision of the *Schistidium apocarpum* complex in Norway and Sweden.
1996	Jorun Skjærmo	Dr. scient Botany	Microbial ecology of early stages of cultivated marine fish; inpact fish-bacterial interactions on growth and survival of larvae.
1996	Ola Ugedal	Dr. scient. Zoology	Radiocesium turnover in freshwater fishes
1996	Ingibjørg Einarsdottir	Dr. scient. Zoology	Production of Atlantic salmon (*Salmo salar*) and Arctic charr (*Salvelinus alpinus*): A study of some physiological and immunological responses to rearing routines.
1996	Christina M. S. Pereira	Dr. scient. Zoology	Glucose metabolism in salmonids: Dietary effects and hormonal regulation.
1996	Jan Fredrik Børseth	Dr. scient. Zoology	The sodium energy gradients in muscle cells of *Mytilus edulis* and the effects of organic xenobiotics.

1996	Gunnar Henriksen	Dr. scient. Zoology	Status of Grey seal *Halichoerus grypus* and Harbour seal *Phoca vitulina* in the Barents sea region.
1997	Gunvor Øie	Dr. scient Bothany	Eevalution of rotifer *Brachionus plicatilis* quality in early first feeding of turbot *Scophtalmus maximus* L. larvae.
1997	Håkon Holien	Dr. scient Botany	Studies of lichens in spurce forest of Central Norway. Diversity, old growth species and the relationship to site and stand parameters.
1997	Ole Reitan	Dr. scient. Zoology	Responses of birds to habitat disturbance due to damming.
1997	Jon Arne Grøttum	Dr. scient. Zoology	Physiological effects of reduced water quality on fish in aquaculture.
1997	Per Gustav Thingstad	Dr. scient. Zoology	Birds as indicators for studying natural and human-induced variations in the environment, with special emphasis on the suitability of the Pied Flycatcher.
1997	Torgeir Nygård	Dr. scient. Zoology	Temporal and spatial trends of pollutants in birds in Norway: Birds of prey and Willow Grouse used as Biomonitors.
1997	Signe Nybø	Dr. scient. Zoology	Impacts of long-range transported air pollution on birds with particular reference to the dipper *Cinclus cinclus* in southern Norway.
1997	Atle Wibe	Dr. scient. Zoology	Identification of conifer volatiles detected by receptor neurons in the pine weevil (*Hylobius abietis*), analysed by gas chromatography linked to electrophysiology and to mass spectrometry.
1997	Rolv Lundheim	Dr. scient. Zoology	Adaptive and incidental biological ice nucleators.
1997	Arild Magne Landa	Dr. scient. Zoology	Wolverines in Scandinavia: ecology, sheep depredation and conservation.
1997	Kåre Magne Nielsen	Dr. scient Botany	An evolution of possible horizontal gene transfer from plants to sail bacteria by studies of natural transformation in *Acinetobacter calcoacetius*.
1997	Jarle Tufto	Dr. scient. Zoology	Gene flow and genetic drift in geographically structured populations: Ecological, population genetic, and statistical models

1997	Trygve Hesthagen	Dr. philos. Zoology	Population responces of Arctic charr (*Salvelinus alpinus* (L.)) and brown trout (*Salmo trutta* L.) to acidification in Norwegian inland waters
1997	Trygve Sigholt	Dr. philos. Zoology	Control of Parr-smolt transformation and seawater tolerance in farmed Atlantic Salmon (*Salmo salar*) Effects of photoperiod, temperature, gradual seawater acclimation, NaCl and betaine in the diet
1997	Jan Østnes	Dr. scient. Zoology	Cold sensation in adult and neonate birds
1998	Seethaledsumy Visvalingam	Dr. scient Botany	Influence of environmental factors on myrosinases and myrosinase-binding proteins.
1998	Thor Harald Ringsby	Dr. scient. Zoology	Variation in space and time: The biology of a House sparrow metapopulation
1998	Erling Johan Solberg	Dr. scient. Zoology	Variation in population dynamics and life history in a Norwegian moose (*Alces alces*) population: consequences of harvesting in a variable environment
1998	Sigurd Mjøen Saastad	Dr. scient Botany	Species delimitation and phylogenetic relationships between the Sphagnum recurvum complex (Bryophyta): genetic variation and phenotypic plasticity.
1998	Bjarte Mortensen	Dr. scient Botany	Metabolism of volatile organic chemicals (VOCs) in a head liver S9 vial equilibration system in vitro.
1998	Gunnar Austrheim	Dr. scient Botany	Plant biodiversity and land use in subalpine grasslands. – A conservtaion biological approach.
1998	Bente Gunnveig Berg	Dr. scient. Zoology	Encoding of pheromone information in two related moth species
1999	Kristian Overskaug	Dr. scient. Zoology	Behavioural and morphological characteristics in Northern Tawny Owls *Strix aluco*: An intra- and interspecific comparative approach
1999	Hans Kristen Stenøien	Dr. scient Bothany	Genetic studies of evolutionary processes in various populations of nonvascular plants (mosses, liverworts and hornworts)

1999	Trond Arnesen	Dr. scient Botany	Vegetation dynamics following trampling and burning in the outlying haylands at Sølendet, Central Norway.
1999	Ingvar Stenberg	Dr. scient. Zoology	Habitat selection, reproduction and survival in the White-backed Woodpecker *Dendrocopos leucotos*
1999	Stein Olle Johansen	Dr. scient Botany	A study of driftwood dispersal to the Nordic Seas by dendrochronology and wood anatomical analysis.
1999	Trina Falck Galloway	Dr. scient. Zoology	Muscle development and growth in early life stages of the Atlantic cod (*Gadus morhua* L.) and Halibut (*Hippoglossus hippoglossus* L.)
1999	Torbjørn Forseth	Dr. scient. Zoology	Bioenergetics in ecological and life history studies of fishes.
1999	Marianne Giæver	Dr. scient. Zoology	Population genetic studies in three gadoid species: blue whiting (*Micromisistius poutassou*), haddock (*Melanogrammus aeglefinus*) and cod (*Gradus morhua*) in the North-East Atlantic
1999	Hans Martin Hanslin	Dr. scient Botany	The impact of environmental conditions of density dependent performance in the boreal forest bryophytes *Dicranum majus*, *Hylocomium splendens*, *Plagiochila asplenigides*, *Ptilium crista-castrensis* and *Rhytidiadelphus lokeus*.
1999	Ingrid Bysveen Mjølnerød	Dr. scient. Zoology	Aspects of population genetics, behaviour and performance of wild and farmed Atlantic salmon (*Salmo salar*) revealed by molecular genetic techniques
1999	Else Berit Skagen	Dr. scient Botany	The early regeneration process in protoplasts from *Brassica napus* hypocotyls cultivated under various g-forces
1999	Stein-Are Sæther	Dr. philos. Zoology	Mate choice, competition for mates, and conflicts of interest in the Lekking Great Snipe
1999	Katrine Wangen Rustad	Dr. scient. Zoology	Modulation of glutamatergic neurotransmission related to cognitive dysfunctions and Alzheimer's disease
1999	Per Terje Smiseth	Dr. scient. Zoology	Social evolution in monogamous families: mate choice and conflicts over parental care in the Bluethroat (*Luscinia s. svecica*)

1999	Gunnbjørn Bremset	Dr. scient. Zoology	Young Atlantic salmon (*Salmo salar* L.) and Brown trout (*Salmo trutta* L.) inhabiting the deep pool habitat, with special reference to their habitat use, habitat preferences and competitive interactions
1999	Frode Ødegaard	Dr. scient. Zoology	Host spesificity as parameter in estimates of arhrophod species richness
1999	Sonja Andersen	Dr. scient Bothany	Expressional and functional analyses of human, secretory phospholipase A2
2000	Salvesen, Ingrid	Dr. scient Botany	Microbial ecology in early stages of marine fish: Development and evaluation of methods for microbial management in intensive larviculture
2000	Ingar Jostein Øien	Dr. scient. Zoology	The Cuckoo (*Cuculus canorus*) and its host: adaptions and counteradaptions in a coevolutionary arms race
2000	Pavlos Makridis	Dr. scient Botany	Methods for the microbial econtrol of live food used for the rearing of marine fish larvae
2000	Sigbjørn Stokke	Dr. scient. Zoology	Sexual segregation in the African elephant (*Loxodonta africana*)
2000	Odd A. Gulseth	Dr. philos. Zoology	Seawater tolerance, migratory behaviour and growth of Charr, (*Salvelinus alpinus*), with emphasis on the high Arctic Dieset charr on Spitsbergen, Svalbard
2000	Pål A. Olsvik	Dr. scient. Zoology	Biochemical impacts of Cd, Cu and Zn on brown trout (*Salmo trutta*) in two mining-contaminated rivers in Central Norway
2000	Sigurd Einum	Dr. scient. Zoology	Maternal effects in fish: Implications for the evolution of breeding time and egg size
2001	Jan Ove Evjemo	Dr. scient. Zoology	Production and nutritional adaptation of the brine shrimp *Artemia* sp. as live food organism for larvae of marine cold water fish species
2001	Olga Hilmo	Dr. scient Botany	Lichen response to environmental changes in the managed boreal forset systems
2001	Ingebrigt Uglem	Dr. scient. Zoology	Male dimorphism and reproductive biology in corkwing wrasse (*Symphodus melops* L.)

2001	Bård Gunnar Stokke	Dr. scient. Zoology	Coevolutionary adaptations in avian brood parasites and their hosts
2002	Ronny Aanes	Dr. scient	Spatio-temporal dynamics in Svalbard reindeer (*Rangifer tarandus platyrhynchus*)
2002	Mariann Sandsund	Dr. scient. Zoology	Exercise- and cold-induced asthma. Respiratory and thermoregulatory responses
2002	Dag-Inge Øien	Dr. scient Botany	Dynamics of plant communities and populations in boreal vegetation influenced by scything at Sølendet, Central Norway
2002	Frank Rosell	Dr. scient. Zoology	The function of scent marking in beaver (*Castor fiber*)
2002	Janne Østvang	Dr. scient Botany	The Role and Regulation of Phospholipase A_2 in Monocytes During Atherosclerosis Development
2002	Terje Thun	Dr. philos Biology	Dendrochronical constructions of Norwegian conifer chronologies providing dating of historical material
2002	Birgit Hafjeld Borgen	Dr. scient Biology	Functional analysis of plant idioblasts (Myrosin cells) and their role in defense, development and growth
2002	Bård Øyvind Solberg	Dr. scient Biology	Effects of climatic change on the growth of dominating tree species along major environmental gradients
2002	Per Winge	Dr. scient Biology	The evolution of small GTP binding proteins in cellular organisms. Studies of RAC GTPases in *Arabidopsis thaliana* and
2002	Henrik Jensen	Dr. scient Biology	Causes and consequenses of individual variation in fitness-related traits in house sparrows
2003	Jens Rohloff	Dr. philos Biology	Cultivation of herbs and medicinal plants in Norway – Essential oil production and quality control
2003	Åsa Maria O. Espmark Wibe	Dr. scient Biology	Behavioural effects of environmental pollution in threespine stickleback *Gasterosteus aculeatur* L.
2003	Dagmar Hagen	Dr. scient Biology	Assisted recovery of disturbed arctic and alpine vegetation – an integrated approach
2003	Bjørn Dahle	Dr. scient Biology	Reproductive strategies in Scandinavian brown bears

2003	Cyril Lebogang Taolo	Dr. scient Biology	Population ecology, seasonal movement and habitat use of the African buffalo (*Syncerus caffer*) in Chobe National Park, Botswana
2003	Marit Stranden	Dr.scient Biology	Olfactory receptor neurones specified for the same odorants in three related Heliothine species (*Helicoverpa armigera, Helicoverpa assulta* and *Heliothis virescens*)
2003	Kristian Hassel	Dr.scient Biology	Life history characteristics and genetic variation in an expanding species, *Pogonatum dentatum*
2003	David Alexander Rae	Dr.scient Biology	Plant- and invertebrate-community responses to species interaction and microclimatic gradients in alpine and Artic environments
2003	Åsa A Borg	Dr.scient Biology	Sex roles and reproductive behaviour in gobies and guppies: a female perspective
2003	Eldar Åsgard Bendiksen	Dr.scient Biology	Environmental effects on lipid nutrition of farmed Atlantic salmon (*Salmo Salar* L.) parr and smolt
2004	Torkild Bakken	Dr.scient Biology	A revision of Nereidinae (Polychaeta, Nereididae)
2004	Ingar Pareliussen	Dr.scient Biology	Natural and Experimental Tree Establishment in a Fragmented Forest, Ambohitantely Forest Reserve, Madagascar
2004	Tore Brembu	Dr.scient Biology	Genetic, molecular and functional studies of RAC GTPases and the WAVE-like regulatory protein complex in *Arabidopsis thaliana*
2004	Liv S. Nilsen	Dr.scient Biology	Coastal heath vegetation on central Norway; recent past, present state and future possibilities
2004	Hanne T. Skiri	Dr.scient Biology	Olfactory coding and olfactory learning of plant odours in heliothine moths. An anatomical, physiological and behavioural study of three related species (*Heliothis virescens, Helicoverpa armigera* and *Helicoverpa assulta*).
2004	Lene Østby	Dr.scient Biology	Cytochrome P4501A (CYP1A) induction and DNA adducts as biomarkers for organic pollution in the natural environment

2004	Emmanuel J. Gerreta	Dr. philos Biology	The Importance of Water Quality and Quantity in the Tropical Ecosystems, Tanzania
2004	Linda Dalen	Dr.scient Biology	Dynamics of Mountain Birch Treelines in the Scandes Mountain Chain, and Effects of Climate Warming
2004	Lisbeth Mehli	Dr.scient Biology	Polygalacturonase-inhibiting protein (PGIP) in cultivated strawberry (*Fragaria* x *ananassa*): characterisation and induction of the gene following fruit infection by *Botrytis cinerea*
2004	Børge Moe	Dr.scient Biology	Energy-Allocation in Avian Nestlings Facing Short-Term Food Shortage
2005	Matilde Skogen Chauton	Dr.scient Biology	Metabolic profiling and species discrimination from High-Resolution Magic Angle Spinning NMR analysis of whole-cell samples
2005	Sten Karlsson	Dr.scient Biology	Dynamics of Genetic Polymorphisms
2005	Terje Bongard	Dr.scient Biology	Life History strategies, mate choice, and parental investment among Norwegians over a 300-year period
2005	Tonette Røstelien	Dr.scient Biology	Functional characterisation of olfactory receptor neurone types in heliothine moths
2005	Erlend Kristiansen	Dr.scient Biology	Studies on antifreeze proteins
2005	Eugen G. Sørmo	Dr.scient Biology	Organochlorine pollutants in grey seal (*Halichoerus grypus*) pups and their impact on plasma thyrid hormone and vitamin A concentrations.