DECODING THE LANGUAGE OF HYPOGLOSSAL MOTOR CONTROL

by

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SIGNED: Christopher Laine
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ABSTRACT

To effect movement, the central nervous system must appropriately coordinate the activities of pools of motoneurons (MNs), the cells which control muscle fibers. Sources of neural drive are often distributed to many MNs of a pool, and thus can synchronize the activities of targeted MNs. In this thesis, synchronization among MNs is used to investigate the strength, temporal progression, and anatomical distribution of neural drive to the hypoglossal motor nucleus (HMN), which controls muscles of the tongue. The HMN is an ideal target for such an investigation because it processes a host of functionally diverse inputs, such as those related to breathing, speaking, and swallowing. Study 1 characterizes motor unit (MU) synchronization within and across bellies of the human genioglossus (GG) muscle when MUs are activated by cortical drive (during voluntary tongue protrusion) or by automatic, brainstem-mediated drive (during rest breathing). We show that voluntary tongue protrusion synchronizes MU spike timing and firing rates within but not across bellies of the GG, whereas during rest breathing, MU firing rates are moderately synchronized both within and across muscle bellies. Study 2 documents respiratory-related synchronization of MU activities in muscles of the tongue and respiratory pump using an anesthetized rat model. The results of this study indicate that upper airway and respiratory pump MN pools share a low frequency respiratory-related drive, but that higher frequency (> 8 Hz) synchronization is strongest in MU pairs of the chest-wall. Finally, Study 3 examines the potential for GG multi-unit and single MU activities to be entrained by cortical input. We show that during voluntary
tongue protrusion, cortical oscillations in the 15-40 Hz range weakly synchronize MU population activity, and that EEG oscillations in this range intermittently influence the spike timing of individual GG MUs. These studies are the first to characterize MU synchronization by different sources of neural input to the HMN and establish a broad foundation for further investigation of hypoglossal motor control.
INTRODUCTION

Study goals and general context

The focus of this thesis is to better understand the basic elements of neuromuscular control of the tongue. Of fundamental importance to this work is the concept that neural inputs to individual motoneurons (MNs) or populations of MNs leave identifiable fingerprints in their output activity. More specifically, inputs shared by groups of MNs will influence the activities of each MN simultaneously, thus synchronizing their output. Therefore, synchrony between MNs, or between MNs and a source of neural input (entrainment), contains information about the strength, temporal progression, and even anatomical distribution of that input (for reviews see Farmer et al., 1997; Farmer, 1998; Funk and Parkis, 2002; Grosse et al., 2002). For the most part, analysis of synchrony has been applied to spinal MN pools and to the investigation of limb control; the approach has not commonly been applied to the investigation of cranial MN pools, especially with regard to lingual/oromotor control.

This work begins with a review of literature pertaining to 1) basic elements of motor control, in particular, factors influencing activation of individual motor units (MUs) and their utilization as members of a pool 2) the methodology, application, and interpretation of several types of synchronization measures, including short-term synchronization, common drive, and coherence analysis, and 3) the functional and anatomical circuitry of the HMN. Following the review, the methodology and findings of three complete studies will be summarized in the chapter entitled “Present Study”,
which also includes a section outlining future avenues of investigation that arise from the studies.

The three studies included in this document (see Appendices A-C) each report on one or more aspects of synchronization among hypoglossal MNs as elicited by voluntary or involuntary neural drives. All three studies have been accepted for publication in the Journal of Neurophysiology.

By way of a brief overview, Study 1 represents the first general characterization of synchrony among hypoglossal MUs during rest breathing and volitional tongue protrusion. My contributions to this project included specifics of the experimental design, selection of the analytical methods used, collection of the data, and authorship of the manuscript. Study 2 resulted from a collaborative project characterizing MN synchronization across respiratory motor pools of the chest-wall and upper airway (including hypoglossal) in an anesthetized rat model. My contributions to this project include analysis and interpretation of the data and conceptual contributions to the resultant manuscript. Study 3 examines the influence of high frequency oscillatory cortical drive on human hypoglossal MUs using both a standard synchrony analysis and a novel method designed to track the effects of cortical drive on the discharge timing of individual MUs (as distinct from synchrony between MUs of a pool). My individual contributions to this project include the design and execution of the experiments, analysis/interpretation of the data, and authorship of the resultant manuscript.
Literature Review

General principles

Electromyographic signals (EMG) contain a great deal of information about the strength and patterning of neural drive to muscles, as well as the neural circuitry which delivers this drive. The research presented in this thesis deals with specific features of the EMG signals that may be of use in probing aspects of neuromuscular control of the tongue. What follows is a brief review of the key elements of motor control, with some basic details regarding the physiology underlying patterns of EMG activity.

i. Motoneurons, motor units, and EMG

The basic element of motor control is the motor unit (MU), which consists of an alpha-motoneuron and the muscle fibers which it (uniquely) innervates (Liddell and Sherrington, 1925). Action potentials fired by the MN cause contraction of the innervated muscle fibers and a corresponding sharp fluctuation in voltage that can be detected by an electrode in the muscle belly or on the skin overlying the muscle. This voltage fluctuation is called a motor unit action potential (or “spike”).

Low impedance intramuscular or surface electrodes detect a summation of activity across a large area of muscle, (hence the term “whole muscle” activity) whereas higher impedance electrodes detect the activities of one or a few MUs, with minimal contamination from adjacent muscle fibers. In any EMG signal (but especially in the latter type of recording), the spikes of a single MU can be isolated by sorting all
identifiable spikes according to their shape and amplitude. The shape of a spike from a single MU depends on the proximity and orientation of the muscle fibers with respect to the recording site, and thus by grouping spikes according to their shape and amplitude, the activity of individual MUs (and thus motoneurons) can be discriminated. This is particularly convenient in studies involving human subjects because the cell bodies of motoneurons are located in nuclei within the brainstem or spinal cord, greatly limiting access to them. Since whole muscle EMG signals represent a population measure of motoneuron activation, fluctuations in the amplitude of whole muscle EMG reflects fluctuations in the activity patterns of many MUs. The key determinants of whole muscle EMG amplitude include the number of MUs recruited, their firing rates, and their synchronization with one another (reviewed in Farina et al., 2004). In the following section, I will briefly review the factors which influence the recruitment of MUs as well as determine their firing rates. Synchronization of MU activities will be addressed in detail in sections B and C.

ii. Activation of motoneurons

Early studies showed that larger motoneurons have lower input resistances than smaller ones, and thus the same current step will evoke a smaller voltage change in a large cell vs. a small one, in accordance with Ohm’s law (Henneman, 1957; Henneman et al., 1965a, 1965b). This means that as excitation to a pool of motoneurons increases overall, the small motoneurons are recruited first and the largest last, an observation now referred to as the “size principle” (Henneman, 1957; Henneman et al., 1965a, 1965b).
Additionally, the larger MNs typically innervate a greater number of muscle fibers (Eccles and Sherrington, 1930) and display larger twitch tension (Milner-Brown et al., 1973a) and tetanic force (Mizote, 1982) than smaller MNs, although there are typically fewer of them in a motor pool overall (Wuerker et al., 1965; Milner-Brown et al., 1973b). Once recruited, the firing rate of a motoneuron will increase fairly linearly with increasing current (Granit et al., 1963a, 1963b; Schwindt and Crill, 1982). At a given level of force output from the muscle, the firing rates of earlier recruited (i.e. smaller, lower threshold) motor units are higher than the later recruited ones, a property termed as the “onion skin” phenomenon (De Luca et al., 1982a, 1982b; De Luca and Erim, 1994; De Luca and Hostage, 2010). The firing rate of a newly recruited motoneuron may be partly determined by the duration of a post-spike decrease in excitability, or “afterhyperpolarization” (Kernell, 1965) in addition to other intrinsic properties discussed in the next section. In general, firing rates at recruitment are similar for all MUs of a muscle (Monster and Chan, 1977), but in some cases may correlate with recruitment threshold (Barry et al., 2007).

The response of motoneurons to current input is both dynamic and flexible. For example, motor unit firing rates can decline in the face of a constant current injection (Kernell and Monster, 1982; Sawczuk et al., 1995; Powers et al., 1999), and this “firing rate adaptation” is attributed to inactivation of sodium channels (Powers et al., 1999). Further, a brief stimulus can produce a period of self-sustained firing due to a “plateau potential” (Hultborn et al., 1975; Schwindt and Crill, 1980; Hounsgaard et al., 1984). Subsequent studies of this phenomenon have led to the identification of voltage activated,
neuromodulator-dependent calcium and sodium persistent inward currents (PICs) that can modify the slope of the firing frequency-input current relationship. PICs may influence the minimal firing rate of motoneurons and even provide the main depolarizing current for motoneurons (reviewed in Heckmann et al., 2005 and Brownstone, 2006).

Physiologically, however, motoneurons are not driven by constant currents but by dynamic ones, which has led to investigation of motoneuron activities driven by time-varying currents. For example, more current is necessary to bring a motoneuron to spiking threshold if that current is injected slowly than for a fast current injection. This phenomenon is referred to as “spike-threshold accommodation” (Araki and Otani, 1959), and has been attributed to the dynamics of sodium channel inactivation (Vallbo, 1964). Further, recruitment thresholds vary as a function of task (Desmedt and Godaux, 1977a, 1977b; Loeb, 1985; Butler et al., 1999). For example, more MUs are recruited per force level in a fast contraction vs. a slow one (Desmedt and Godaux, 1977a, 1977b). In addition, recruitment thresholds can be modified by sensory feedback (Garnett and Stephens, 1981). Finally, it has been shown that many types of neurons (including motoneurons) react to oscillatory inputs in a frequency dependent manner, responding more efficiently (i.e., higher current-to-voltage gain and with greater temporal precision) to input delivered at an intrinsically “preferred frequency” of the cell (Hunter et al., 1998; Fellous et al., 2001; Funk and Parkis, 2002; Parkis et al., 2003; van Brederode and Berger, 2008). In summary, while the intensity and temporal progression of input current will be reflected in the firing of the motoneuron, the transfer from input to output depends on the dynamics of both the input current and the intrinsic membrane properties of the cell.
iii. Utilization of motoneurons within a pool

Both the number of active MUs (recruitment) and their firing rates (rate coding) influence overall muscle force (Adrian and Bronk, 1929). The relative contribution of each mechanism to the generation of force is determined by several factors. For one, the strategy of force generation varies depending on specifics of the muscle/motor pool. For example, all MUs of the adductor pollicis muscle are recruited at forces less than 50% of maximal voluntary contraction (MVC) (Kukulka and Clamann, 1981), while some MUs of the biceps brachii can be recruited at force levels beyond 80% MVC (Kukulka and Clamann, 1981). Recruitment may be favored at lower force levels requiring activation of only a small proportion of MNs in a pool (Milner-Brown et al., 1973b) and during slow contractions at low force levels (Heckman and Binder, 1991; Fuglevand et al., 1993). Recruitment may also be important for powerful contractions that do not require a great deal of precise force control (Adrian and Bronk, 1929; Kukulka and Clamann, 1981) although rate coding may contribute significantly once a high proportion of the MN pool is already active (Milner-Brown et al., 1973c; Fuglevand et al., 1993). Rate coding may be particularly important when precise control is required, for example, in small muscles of the hand (de Luca et al., 1982b). Finally, synchronization of MUs with each other on various time scales can also impact force and EMG amplitude on a modest scale (de Luca et al., 1982b; Yao et al., 2000). The characteristics of MU synchronization do, however, reveal a great deal of information about the temporal progression, timescale, and even
physical distribution of synaptic drive onto motor pools, as will be discussed in the next section.

Synchronous activity between pairs of single motor units

As mentioned earlier, control of a motor pool depends not only upon the number and firing rate of active cells, but also on the coordination of MNs across the pool. In this section, three types of MU-MU synchronization will be reviewed, namely, short-term synchrony, common drive, and coherence. Discussion of each type of synchronization will include the physiological interpretation/origin of the phenomenon, the methodology used to detect it, as well as a brief overview of the experimental factors known to influence its strength.

Short-term synchrony:

i. Physiological interpretation/origin

One of the earliest identified and most well-studied types of synchronization is the scenario in which two MNs display a weak tendency to fire action potentials simultaneously with each other (Moore et al., 1966). In this case, ‘simultaneously’ refers to action potentials occurring within about $\pm 3 - 5$ ms of each other (Sears and Stagg, 1976; Kirkwood et al., 1982). The occurrence of simultaneous spiking between two MUs is often more frequent than would be expected by chance, and is thought to occur when different MNs receive input from axon branches of the same premotor neurons (Datta and Stephens, 1990). Premotor drive delivered in this way will tend to depolarize both MNs
at the same time, leading to an increased probability that they will fire at the same time as well. The degree to which shared axonal input would cause simultaneous firing between two MNs depends upon several factors, including the density and relative strength of ‘shared’ input compared with other inputs (Lemon, 1993). The degree of short-term synchronization can therefore quantify the strength of shared axonal input across a motor pool. This type of analysis can help identify the presence of branched projections and/or their functional relevance under different experimental conditions.

It is important to note that the temporal definition of ‘simultaneous’ is not arbitrary. Kirkwood and colleagues (Kirkwood et al., 1982) described two other forms of MU-MU synchronization, each of which has a slightly different physiological interpretation. The first refers to the scenario in which two MNs fire within a short time period of each other more often than expected by chance, but for time periods more than 4 fold larger than that used to define short-term synchronization. This type of coordination was called ‘broad-peak’ synchronization in reference to the cross-correlation analysis used to identify synchronous spiking (see next section). The physiological origin of broad-peak synchrony is thought to be synchronization of premotor neurons, which then send their projections downstream to separate MNs, two of which are recorded from (Kirkwood et al., 1982; Murthy and Fetz, 1996; McAuley et al., 1997). From MU-MU correlation analysis, it is not clear if premotor synchronization is due to shared axonal input from neurons further upstream, or some other synchronizing force. The second type of phenomenon identified was periodic synchronization among MUs. This type of
synchronization is better detected using coherence analysis, and will be described further in the review of coherence analysis.

ii. Methodology

The quantification of short-term synchrony is based around the application of cross-correlation analysis to the spike trains of two simultaneously recorded MUs. In general, a correlation measures similarity between two signals. In many cases, one signal may lead or lag behind another in time. In order to more fully describe the relationship between the two signals, their correlation can be calculated across a range of lags, or in other words, one can calculate a cross-correlation. In the context of measuring synchronization between MUs, correlation strength at small lags is taken to represent the degree of simultaneous spiking between the MUs while correlation at large lags is used to represent the degree of simultaneous firing expected by chance. For example, correlation at lags of 40-100 ms have been used to represent chance-level (Keen and Fuglevand, 2004) since it is unlikely that there should be any consistent relationship in the spike timing of two MUs across such a large time span. In analyzing MU-MU synchronization, the cross-correlation function is applied to a processed representation of MU activity rather than voltage recordings. Motor unit action potentials are first grouped according to their shape and amplitude in order to identify and isolate spikes from single motor units. An example of two motor unit spike trains is shown in Figure 1 (top), with the action potentials of each shown overlaid in the inset. The spike times of each individual MU are then processed into binary pulse trains, assigning to each ms of recording either a
1 (indicating a spike occurred at this time), or a 0 (indicating no spike at this time). This allows the cross-correlation analysis to be conducted on spike times rather than spike-containing signals, thereby targeting spike-timing relationships exclusively. When analyzing continuous signals, the correlation values calculated for each lag are typically normalized to obtain an index of linear correlation with values falling between -1 and 1, with 1 indicating a perfect linear relationship and 0 indicating no linear relationship between the signals. In the context of motor control, this is not typically done, and instead, the output of the cross-correlation analysis is a histogram (a cross-correlogram) which indicates how often the two MUs fired action potentials with a given time-offset (lag) from each other. An example can be seen in figure 1 (bottom), which shows a cross-correlogram of 2 MUs recorded from the human genioglossus during voluntary tongue protrusion. Note that the Y axis of the cross-correlogram is simply a count of the number of spikes that occurred in one MU at a given lag with respect to the timing of spikes fired by the other MU. It is important to point out that the actual number of simultaneous spikes counted across a recording is of little value, and depends upon factors such as the recording duration and the firing rates of the MUs, as well as the actual strength of common input to both.

The procedure for converting histogram bin counts to a useful index of short-term synchronization strength has not been standardized, and several methods have been proposed. Perhaps the simplest method is to calculate the K index, which is the value of the largest histogram bin (within a few ms of time 0) divided by the mean bin count across a range of large lags representing chance level (Sears and Stagg, 1976). Given
that the width of a cross-correlogram peak can be important in interpreting how common input is delivered physiologically (Kirkwood et al., 1982), most researchers now take into consideration both the height and width of a ‘peak region’. A method that has been widely adopted for determination of the ‘peak region’ is the cumulative sum derivative of the cross-correlogram (Ellaway, 1978). The peak-region can be determined manually by the investigator, or more quantitatively, designated as the points where the cumulative sum derivative traces exceed certain thresholds, such as 10 and 90 percent of the difference between maximum and minimum values (Schmied et al., 1993). To calculate an index of short-term synchronization strength that takes the shape of the cross-correlogram peak into account, the K’ index (Ellaway and Murthy, 1985; Nordstrom et al., 1990) has been used and is defined as the total counts (synchronous spikes) in the peak region of the cross correlogram divided by an off-peak mean bin count. An example application of the CUSUM procedure is shown in figure 1b, where it is used to define the peak-region necessary for calculation of a K’ value. Another common measure is the SI index, the calculation of which involves subtracting the mean off-peak bin count from the total counts in the peak-region (providing the number of ‘extra’ simultaneous spikes) and then dividing by the total counts in the cross-correlogram (Logigian et al., 1988). The S index is the same as the SI index other than that the number of ‘extra counts’ in the peak region is divided by the total number of spikes fired by both MUs rather than the number of counts in the cross-correlogram (Adams et al., 1989). Similarly, the E index divides the ‘extra counts’ in the peak region by the number of spikes fired by only the reference unit (Datta et al., 1991). Finally, Nordstrom et al.
developed the CIS index, which divides the ‘extra counts’ in the peak region by the
duration of the recording (Nordstrom et al., 1992). The later method is not influenced by
the firing rates of motor units as the other indices are (Nordstrom et al., 1992), however,
it may not be suitable (without modification) in cases where MU firing is not continuous
across a recording. The preponderance of methods used can complicate comparison
across studies, since synchronization may be expressed as a % above chance-level (as
derived from the K’ index) in one study, and in another, as the rate of ‘extra’
simultaneous spiking (as in the CIS index).

Since all of these methods require a ‘peak-region’ to be identified, their efficacy
would be greatly hampered by the absence of a peak. Determination of whether or not a
peak actually exists in a cross-correlogram can be accomplished by statistically
comparing off-peak bin counts with those of a potential peak region (e.g. a z-score). If a
significant peak is not identified, an 11 ms window centered at time 0 can be used as the
default ‘peak-region’, so that subtle changes in synchronization strength can be evaluated
(Semmler and Nordstrom, 1995a). It should be noted that nearly all measures of short-
term synchronization rely to some degree on a ‘chance-level’ derived from the larger lag
times in a cross-correlogram. Because of this, application of these techniques to MUs
with patterned firing behavior (e.g. bursting of respiratory-related MUs) or which receive
oscillatory common input must be attempted carefully. This type of activity complicates
the determination ‘chance-level’ since no flat baseline period is present within the cross-
correlogram.
iii. Experimental findings

While the phenomenon of short-term synchrony is a useful assay with which to probe neural drive, the functional relevance of synchronization itself is not entirely clear. Simulations have shown that synchronization of MUs can increase EMG amplitude and fluctuations in force, but not the average force level produced by a muscle (Yao et al., 2000). In contrast, higher force contractions are associated with higher levels of short-
term synchronization between MUs (Fling et al., 2009). In that sense, it may be useful to understand short-term synchronization as a tool for investigating the strength of certain types of synaptic drive rather than as the intended result of that drive. To that end, researchers have focused on short-term synchronization as an assay of neural drive under different experimental conditions. For example, sternocleidomastoid MUs show the strongest synchronization during voluntary obstructed breathing vs. isometric neck rotation or reflex hypercapnic breathing (Adams et al., 1989). Bremner et al., (1991) showed that MUs of the first dorsal enteroosseous (FDI) display greater synchronization during finger extension than flexion or abduction. Also, even when the task/degree of muscle contraction is held constant, the level of attention paid to that task can influence the strength of short-term synchrony (Schmied et al., 2000). From studies of short-term synchronization in FDI MUs of strength and skill trained subjects, it was suggested that skill-training may decrease its strength and strength training may increase it (Semmler and Nordstrom, 1998). However, short-term strength training (4-8 weeks) does not produce any differences in FDI synchronization (Kidgell et al., 2006), which suggests that the effect of training in earlier studies may have been more related to plasticity in the delivery of cortical drive rather than the strength of the muscle per se. Along similar lines, differences in the magnitude of short-term synchronization found in the right and left hands have been found to be influenced by handedness (Schmied et al., 1994; Semmler and Nordstrom, 1995b).

Proprioceptive feedback may not have a great deal of influence on the level of short-term synchronization. For example, Garland and Miles (1997) saw no change in
the synchronization level of flexor digitorum profundus (FDP) MUs when the muscle was functionally disengaged from the distal interphalangeal joint of the fourth or third finger. Also, fatigue may not influence the strength of short-term synchrony. Contessa et al., (2009) found little influence of fatigue on short-term synchronization of MUs recorded from the vastus lateralis, and Nordstrom et al., (1990) found no change in synchronization of MUs of the Masseter during prolonged contraction. Short-term synchrony may also be resistant to certain changes in the temporal patterning of descending drive, such as those associated with Parkinson’s disease (Baker et al., 1992). Finally, motor unit synchronization appears to be unaffected by age after maturity has been reached (Kamen and Roy, 2000; Semmler et al., 2006), suggesting stability of short-term synchrony in the absence of factors which might result in neural plasticity (Semmler and Nordstrom, 1998).

In summary, short-term synchronization is most useful as a way to track the intensity of descending drive onto MUs, and can be influenced by details of the experimental task or by changes in the anatomical/functional delivery of drive secondary to training.

Common drive:

i. Physiological interpretation/origin

A major type of synchronization among active MNs is a phenomenon known as common drive. Simply stated, common drive describes the scenario in which two active motor units show concurrent fluctuations in firing rate over time (De Luca et al., 1982a; De
Luca et al., 1982). As before, the fact that firing rate fluctuations occur concurrently between two motor units speaks to the “common” or “shared” nature of the neural drive responsible. Further, the two recorded units need not have similar mean firing rates for synchronized firing rate fluctuations to be present. An example of two such modulated MU firing rate traces are shown in figure 2 (top). As with short-term synchrony analysis, quantification of this phenomenon is accomplished through cross-correlation analysis, and ‘concurrent’ implies a maximal correlation between signals at lags less than 100 ms. Although in concept, common drive covers firing rate fluctuations at almost any timescale, the most standard methodology for quantifying common drive is focused on fluctuations whose peaks and valleys occur on a time-scale similar to sinusoidal oscillations of 1-5 Hz (Myers et al., 2004). Unlike short-term synchrony, there is no clear anatomical origin for common firing rate fluctuations among MUs. It is thought that the direct axonal projections which underlie short-term synchronization are not also the origin of common drive (Semmler et al., 1997; Negro et al., 2009), although under certain conditions, the strength of both measures can be moderately correlated (Semmler et al., 1997). The phenomenon is so widespread across MU pools that when common drive has been identified, it is typically seen in nearly all motor unit pairs recorded.

ii. Methodology

The quantification of common drive is calculated in the time domain using a fairly straight-forward procedure. First, the two single MU recordings are converted to
binary spike trains, as described earlier. Subsequently, each binary spike train is converted to a continuous firing rate trace via convolution with a Hann window. This is nothing more than a moving average where greater weight is assigned to data in the middle of the moving window than at the ends. A standard moving average uses a rectangular window and would work almost as well, but since the first reports of common drive were quantified using a Hann window, the technique has become standard. The width of the moving window is somewhat arbitrary and depends on the firing rates of the motor units as well as the expected time-scale of shared firing rate fluctuations. This issue was specifically addressed in Myers et al., (2004), where it was concluded that the standard 400ms moving Hann window used in most literature will primarily identify firing rate fluctuations in the 1-5 Hz range, while a 200 ms window will be able to resolve faster fluctuations, up to about 10 Hz. The firing rate traces are then high-pass filtered with a cutoff of 0.75 Hz to remove any slow trends that any DC offset between the signals. Finally the two firing rate traces are subjected to a standard cross-correlation analysis and the largest peak within ± 100 ms of 0 lag is identified. This time window is somewhat arbitrary but is reasonable given that average inter-spike-intervals fall between 50 and 100 ms for most MUs (corresponding to 20 and 10 Hz firing rates, respectively). An example of this analysis is shown in Figure 2 (lower trace). The MU pair analyzed is the same pair as depicted in Figure 1 (top). This pair of MUs shows a high degree of common drive, indicated by the peak near the 0 lag time.
iii. Experimental findings

The synchronized fluctuations in MU firing rates that are the hallmark of common drive appear to underlie concurrent fluctuations in muscle force (De Luca, 1985).
Determining the relevance of these force fluctuations is not straightforward given their small magnitude and the fact that common drive is not greatly influenced by the absolute level of force generated by a muscle (De Luca et al., 1982). In this sense, common drive is not necessarily reflective of the intensity of neural input. Unlike measures of short-term synchronization, fatigue and proprioceptive feedback influence the strength of common drive (Contessa et al., 2009; Garland and Miles, 1997). In fatiguing muscles, common drive strength increases, and this has been suggested to stem from a decreased sensitivity of MUs to proprioceptive feedback from spindles (Contessa et al., 2009). The decrease in spindle activity during fatigue has been reported by Macefield et al., (1991), and decreased proprioception/spindle activity has been linked with increased common drive strength (De Luca et al., 2008). In the study of Kamen and De Luca, (1992), common drive was measured in MUs of the orbicularis oris muscle, which lacks muscle spindles. Not only was common drive still present, but the levels of common drive were some of the highest measured for any muscle.

One emerging theme in the literature regarding common drive is that it appears to be larger when gross/automatic activity of a muscle is called for and smaller when fine control is required. For example common drive was found to be stronger when MUs of the Soleus muscle were used for postural control than when the muscle was voluntary contraction (Mochizuki et al., 2006). Similarly, FDI MUs show common drive to be stronger after cerebellar stroke (Sauvage et al., 2006), and reduced in skilled musicians (Semmler and Nordstrom, 1998). In addition, common drive has been measured across synergist muscles (De Luca and Mambrino, 1987), suggesting that common drive may
allow large groups of MUs to be utilized as a functional unit. Like short-term synchronization, short term (8 week) resistance training does not alter the strength of common drive (Beck et al., 2011).

MU-MU Coherence:

i. Physiological interpretation/origin

It is widely understood that rhythmic activity plays a fundamental role in the encoding and transfer of information throughout the nervous system (Singer and Gray, 1995; Basar et al., 2000; Buzsaki, 2006). Quantification of oscillatory activity and oscillatory synchronization between brain areas has become an invaluable tool for researchers seeking to understand how information is transferred between neurons or networks of neurons, and it is of no surprise that this type of analysis has been applied to motor systems as well. At the heart of such research is Fourier analysis, which describes a signal (e.g. spike train, EEG, EMG, etc.) in terms of its correlation with a set of sinusoids covering a range of frequencies. From a mathematical standpoint, it is possible to deconstruct an arbitrary signal as the algebraic summation of sinusoids of varying frequencies, amplitudes, and phases (the relative alignment of each oscillation’s peaks and valleys with each other). In neural data, oscillatory components of signals often have tractable anatomical origins and distinct physiological functions (Farmer, 1998; Funk and Parkis, 2002; Grosse et al., 2002). In the case that two MNs share input containing an oscillatory component, the oscillation will be simultaneously reflected in the firing activity of each MN. To quantify this, a correlation technique that can identify
synchronization between signals on a per-frequency basis is called for. The method, called coherence analysis, is literally a frequency-domain extension of the standard Pearson’s correlation coefficient (a derivation can be found in Roach and Mathalon, (2008)).

In the case of spike trains, the interpretation of coherence varies depending on two major factors: 1) the frequency of coherent activity and 2) how this frequency relates to the firing rates of the motor units. Low frequency coherence (i.e., coherence at frequencies well under the MU firing rates) reflects synchronization of firing rate fluctuations. In fact, coherence over the range of ~1-5 Hz is essentially the same as the time domain common drive measure previously discussed (Myers et al., 2004). The lowest coherent frequencies (<2Hz) found between two MU spike trains are of unknown origin, but are present following capsular stroke (Farmer et al., 1993), making a cortical origin for this common input unlikely. Slightly higher frequencies within the range of common firing rate modulation are decreased following stroke (Farmer et al 1993), suggesting at least some cortical contribution. For higher frequencies of coherence, the physical interpretation can no longer be that of concurrent firing rate fluctuations. Intuitively, for there to be a change in firing rate, two or more inter-spike-intervals must occur. Therefore, the fastest firing rate fluctuations in a signal must be well under the MU firing rate. This fact is often overlooked when coherence is described as a linear correlation between the frequency components of each signal. Recently, the fact that single motor unit spike trains undersample the frequency content of their input has received both empirical and theoretical validation (Negro and Farina, 2011a, 2011b).
The major mechanism through which pairs of MUs can become coherent at frequencies higher than their individual firing rates is the precise timing of simultaneously fired action potentials with respect to a hypothetical stationary oscillation. This implies a link between the degree of short-term synchrony and high frequency coherence. Terry and Griffin, (2008) provide simulation data which supports the idea that a 15-30 Hz common input not only increases the degree of MU-MU coherence at that frequency, but also increases the degree of short-term synchrony. The importance of high frequency coherence in the beta (15-30 Hz) and gamma (30-60 Hz) band is its association with cortical descending drive, which is supported by studies of synchronization between motor unit/EMG activity and cortical activity during voluntary muscle contraction (for reviews see (Brown et al., 1998; Mima and Hallett, 1999; Schnitzler et al., 2000; Grosse et al., 2002)). Both simulations and experimental data support a link between beta/gamma band coherence and short-term synchronization (Farmer et al., 1997; Halliday et al., 1999; Kilner et al., 2002; Semmler et al., 2002, 2004; Lowery et al., 2007), but it should be noted that while coherence at these frequencies may require some degree of short-term synchronization, the opposite is not true, since simultaneous action potentials occurring randomly over time will not be detected in coherence analysis. An alternative explanation for high frequency coherence among spike trains has also been proposed. Moritz (2005) concluded from simulation studies that short-term synchrony, regardless of the timing of simultaneous events, can cause 16-32 Hz coherence. Their derivation, however, relies on a procedure in which a subset of near simultaneous spikes (up to ~30 ms of lag) were pushed into perfect synchrony with each other. This creates a
ripple in the cross-correlation histogram between the two spike trains, and produces coherence at a frequency with a period length equal to the adjustment time. While intriguing to consider a non-oscillatory mechanism by which high frequency coherence can be obtained, such a mechanism would still not explain how EEG-EMG coherence is produced, and thus, 16-32 Hz coherence likely reflects a common oscillation.

ii. Methodology

The most commonly applied coherence analysis is to calculate the “magnitude-squared coherence” between two signals. The magnitude-squared coherence is the square of something called ‘coherency’, which is a complex number. The advantage of describing signal correlations using complex numbers is that phase relationship between signals can be determined in addition to the magnitude of correlation. Phase information has been applied within the realm of motor control to determine time-delays between EEG and surface EMG activity (reviewed in Mima and Hallett, 1999).

Terminology aside, the standard procedure for calculating a frequency-domain correlation between spike trains is given by the following equation:

\[ C_{xy}(f) = \frac{|S_{xy}(f)|^2}{S_{xx}(f)S_{yy}(f)} \]

Where \( C_{xy}(f) \) is the magnitude squared coherence, \( S_{xx} \) and \( S_{yy} \) are the autospectral density functions of signals \( x(t) \) and \( y(t) \), and \( S_{xy}(f) \) is the cross-spectral density function between \( x(t) \) and \( y(t) \). The above equation is an oversimplification in that real world
coherence analysis typically requires that the data be segmented in time, thus requiring several choices to be made regarding segment size, overlap, or weighting (Rosenberg et al., 1989). This weighting (i.e., tapering) is when each segment is multiplied by a weighting function that reduces the signal amplitude at the beginning and end of each segment. This procedure is helpful in that signals are rarely exactly the same at the beginning and end of each segment, however, such symmetry is assumed in standard Fourier analysis, and violations of this assumption can introduce noise to the spectrum. The number of segments used depends on the lowest desired frequency that can be reasonably analyzed within the time period covered by each data segment and also the number of segments desired in total. While the use of overlapping, tapered data segments can lead to cleaner spectral profiles, it can also make determination of the significance of coherence more difficult (Terry and Griffen, 2008). For most research applications, it is sufficient to use un-tapered, non-overlapping (disjoint) data segments, which can then allow for the significance level of coherence to be determined as follows:

\[ P = 1 - \alpha \left( \frac{1}{L-1} \right) \]

Where \( \alpha \) = the % confidence level deemed significant (typically .05) and \( L \) = the number of disjoint data segments used in the coherence analysis (Carter, 1987; Rosenberg et al., 1989). An example coherence profile for a pair of genioglossus MUs is shown in figure 3. The horizontal line marks the 95% confidence level calculated for the recording. It is worth emphasizing that the significance of any coherence value depends...
on how many segments are used in the coherence analysis, and similarly, on whether or not data segments overlap, or are tapered. The practical implication is that coherence values alone are of limited utility unless other details of signal processing methodology are known.

The fact that coherence values are essentially correlation coefficients influences how groups of coherence values should be compared. The measure has a skewed sampling distribution and as such it is technically not correct to use standard parametric statistics to evaluate differences in coherence values without first ‘normalizing’ the coherence values via Fisher’s r-Z transform

\[ Z = \text{atanh}(\sqrt{C}) \]

where C represents the magnitude-squared coherence (Benignus, 1969; Rosenbert et al., 1989). In this case, ‘normalizing’ literally means making the naturally skewed distribution of coherence values expected to be obtained more Gaussian (or normal) so that parametric statistics can be used. In cases where the coherence values were derived from recordings of different time durations, the process of preparing the coherence values for statistical testing must usually include some type of weighting, such as multiplying each Fisher Z value by the number of segments used. Kilner and colleagues multiply each Z value by \(1/\sqrt{2L}\) (Kilner et al., 1999) or \(\sqrt{2L}\) (Kilner et al., 2000), while Amjad et al., (1997) simply produce a weighted average of Z values according to the number of segments used (L) in each recording. The difference is mainly related to the statistical
tests used by these studies to compare coherence values across groups. To obtain an
average coherence value across a group, weighted Fisher Z values can be averaged and
then retransformed to coherence values, or data from multiple recordings can be
concatenated and coherence calculated as for a single recording (Amjad et al., 1997). An
advantage to calculating ‘pooled coherence’ is that significance is calculated in the same
way as for a single recording, whereas a group significance test cannot be conducted on
an average Fisher’s Z-value. An alternative way in which to compare groups of
coherence data is simply to count the percent of recordings showing significant coherence
at a given frequency in each group. This is a straightforward method that has been
standard since the early studies of MU-MU coherence (Farmer et al., 1993; Marsden et
al., 1999), and is often used in parallel with more statistically intensive analyses (e.g.
Halliday et al., 1999; Kilner et al., 2000; Semmler et al., 2002). While coherence
histograms are simple to interpret, they do ignore the actual magnitude of coherence. It
may not always be the case that the probability of recording coherence in MU pairs relays
the same information as the magnitude of their coupling across a group.

Finally, it is important to note that coherence is not strictly a measure of phase-
synchronization (where the peaks and valleys of oscillations are aligned over time), but
also includes the strength of the oscillations within each signal and how they vary over
time with respect to each other. For neural signals, this may be problematic because
evoked changes in oscillatory activity may be observed in either magnitude or phase
relationships. Both types of correlation may be dissociable and serve different functions
(Lachaux et al., 1999; Bruns et al., 2000; Pinto et al., 2003; Makeig et al., 2004). An
An illustrative example of coherence analysis is shown in figure 3 below. The coherence analysis was conducted on the same pair of human genioglossus MUs as in figures 1 and 2. The MU pair depicted showed highly significant coherence at frequencies under 10 Hz.

Figure 3: Example of MU-MU coherence analysis. The top panel shows a 5 s period of discriminated motor unit activity for two simultaneously active GG motor units (same as in figure 1). In the coherence analysis (below), the strength of linear correlation between the two signals at each frequency is shown. The horizontal line marks the 95% confidence level for significant coherence. Figure modified from Laine and Bailey 2011.

iii. Experimental findings

As mentioned earlier, the interpretation of coherence varies depending on frequency, and several frequency bands have been identified which can be differentially manipulated by
task condition or injury (for reviews see Brown 2000; Grosse et al., 2002). The lowest
frequencies of coherence essentially quantify the same phenomenon as time-domain
common drive analysis (Myers et al., 2004), but may reveal information that is lost in the
time domain measure. For example, using coherence analysis, it is possible to
differentiate between a <2 Hz common drive which is not affected by capsular stroke and
a 3-5 Hz band which is abolished by it (Farmer et al., 1993).

During slow movements, MU-MU coherence increases in the 6-12 Hz range
(Vallbo and Wessberg, 1993; Kakuda et al., 1999). In the study of Kakuda et al., (1999),
MU pairs were recorded from the extensor carpi radialis muscle during slow movement
and static position holding. A 6-12 Hz band of coherence between MUs was evident
during movement and was diminished during position holding. Motor unit
synchronization in the frequency range from about 6-12 Hz is thought to reflect the
tremor-causing effects of a circuit oscillation involving spindle feedback (Erimaki and
(<5 Hz) was also observed in wrist MUs, but synchronization in this band was not as
prominent or task-dependent as the 6-12 Hz band coherence.

Perhaps the frequency band that has received the most attention are the beta (15-
30) and gamma (30-60) Hz bands. The degree of MU-MU coherence in this range is
strongly associated with the strength of short-term synchronization, both theoretically
(Moritz, 2005; Lowery et al., 2007) and empirically (Farmer et al., 1993, 1997; Halliday
et al., 1999; Kilner et al., 2002; Semmler et al., 2002, 2004). These investigations, in
combination with studies of coherence between scalp-recorded EEG/MEG and surface
EMG in the same range (Conway et al., 1995; Salenius et al., 1997; Halliday et al. 1998; Brown et al., 1998; Gross et al., 2000), implicate a cortical origin for beta/gamma frequency of common input to MN pools. The relationship to short-term synchronization can be expected given that a high frequency input oscillation must influence the timing of simultaneous spiking between MUs in order to produce coherence between MUs. The simultaneous MU spikes need not occur rhythmically over time, but MU-MU coherence at such high frequencies does require some degree of simultaneous spiking (McAuley and Marsden, 2001). Given this necessity, Kakuda et al., (1999) argued that the relatively low density of corticospinal projections to MNs of the extensor carpi radialis vs. the First Dorsal Interosseous explained why they found little coherence above 15 Hz when it had been a robust feature of recordings from the FDI (Farmer et al., 1993). In this regard, both short-term synchrony and beta/gamma coherence is expected to be lower in MN pools of proximal vs. distal muscles, given their generally lower densities of direct cortical projections (Clough et al., 1968; Lemon, 1993; Marsden et al., 1999). Unlike short-term synchrony, however, simultaneous spiking must be timed appropriately with respect to an input oscillation in order for coherence to be detected, and thus, factors such as synaptic noise or multiple sources of common input may serve to decrease MU-MU coherence at high frequencies, even when the strength of that drive is held constant (Negro and Farina, 2011b).
EEG-EMG Coherence

In the previous section, the effects of common input to MNs were addressed in the context of MU-MU synchronization. When considering the effects of a single source of neural drive (e.g. cortical drive) on MU-MU synchronization, a few conceptual points need to be considered: 1) MU-MU synchronization may stem from a mix of several sources of common input; 2) the effects of individual sources of input must be inferred indirectly from the details of the experimental design and; 3) the presence of multiple sources of synaptic input may weaken the influence of each individual source on MU-MU synchronization (Negro and Farina, 2011a, 2011b). Perhaps the most widely studied ‘individual source’ of input is that of voluntary drive, or more specifically, corticospinal (or corticobulbar) communication to MNs. In cases where a specific source of neural input to a MN pool can be measured, its effects on coordinating/entraining MN activities are quantifiable as the degree of similarity between input and output. The results of this work show cortical oscillations in the beta (15-30 Hz) and gamma (30-50 hz) frequency bands become synchronized with EMG activity during voluntary muscle contraction (Conway et al 1995; Salenius et al 1997; Brown et al 1998; Halliday et al 1998; Gross et al 2000); for reviews see (Mima and Hallet, 1999; Schnitzler et al., 2000; Grosse et al., 2002).

i. Physiological origin/interpretation

For oscillatory components within EEG or MEG signals to be detected, large networks of neurons must show some degree of synchronized activity with each other. Unlike EMG amplitude, which is influenced by the number, size, and location of
individual MUs, changes in EEG amplitude are thought to be largely related to synchronization/desynchronization of large populations of cells (Pfurtscheller and Aranibar, 1977). For example, EEG recordings obtained over sensorimotor cortex show the amplitude of oscillatory activity in the beta range decreasing prior to movement onset (Jasper and Penfield, 1949), and in response to motor imagery (Neuper et al., 1999), but then rebounding shortly after movement stops (Chatrian et al., 1959). Oscillatory activities in the cortex can be transmitted to motor pools directly via pyramidal track neurons (Baker et al., 2003). Corticomuscular coupling in the beta/gamma band (for reviews see Mima and Hallett, 1999; Schnitzler et al., 2000; Grosse et al., 2002) is diminished after pyramidal track lesions (Mima et al., 2001). Studies of the delay (phase lag) between EEG and EMG signals have generally supported the view that corticomuscular coupling results from the entrainment of MU activities by descending cortical drive (e.g. Salenius et al., 1997), however, phase-relationships between EEG and EMG are not necessarily consistent over time or across studies, and it has been speculated that both descending control and peripheral feedback contribute to corticomuscular coupling in the beta band (Riddle and Baker, 2005; Witham et al., 2011).

Mechanistically, the MU-MU coherence that is evoked during voluntary muscle activation (see MU-MU coherence above) must be related to the influence of cortical oscillatory drive on MU spike timing, or more specifically, the timing of simultaneous discharges between MUs. This implies that coherence between EEG and EMG should reflect the same phenomenon as MU-MU coherence. That said, EEG-EMG coherence is a more direct measure of input-output relationships and could be greatly advantageous.
where noise or multiple sources of input might serve to decorrelate spiking output with any single source of input in a given frequency range (Negro and Farina, 2011a, 2011b). In addition, EMG is essentially a population measure, as is EEG, making it more likely that weak or time varying effects on individual MU pairs will be detected in the summation of their activities. The fact that certain effects which may be present in individual MU pairs may not be strongly reflected in average EMG activity, and vice versa is an important reason for measuring both MU-MU and EEG-EMG coherence to obtain a fuller picture of MU control by descending cortical input.

ii. Methodology

Detection of EEG-EMG coherence is similar in many ways to MU-MU coherence analysis in that the exact same coherence function is used, and all the decisions concerning data segmentation, segment overlap, tapering, and concerns about signal stationarity all apply. The major difference relate to the methods used in preparing the EEG and EMG signals for coherence analysis. There is no established standard regarding the most appropriate referencing scheme for EEG recordings, however, a surface Laplacian such as the Hjorth transform (Hjorth, 1975) tends to provide the best spatial resolution (Grosse et al. 2002). The EEG is subsequently filtered to reduce near DC components and high frequency noise. The preprocessing of EMG signals to be used in EEG-EMG coherence analysis has been the subject of recent debate (Myers and O’Malley, 2003; Yao et al., 2007; Boonstra, 2010; Christou and Neto, 2010; Halliday and Farmer, 2010; Neto and Christou, 2010, 2010). Full wave rectification is often used as a
convenient (and always positive) way to express EMG amplitude, but the utility of this procedure in analyzing EEG-EMG or EMG-EMG coherence had not received much consideration prior to the technique becoming standard. Myers and O’Malley (2003) and Yao et al., (2007) have argued that the technique is a valid way to extract the patterns of MU spiking activity from whole muscle EMG. However, Neto and Christou, (2010), have argued on the basis of simulated data that oscillatory common input to MN pools might be better detected using the interference (i.e., unrectified) EMG signal. Boonstra (2010) has defended the utility of rectification and suggested that the findings of Neto and Christou, (2010) were based on an assumption that cortical input at a certain frequency directly affects the amplitude of surface EMG at that frequency, and ignored the possibility that input may comprise periodic bursts or amplitude modulations of higher frequency activity. Halliday and Farmer, (2010) added to the debate, stating that rectification is important in that it demodulates the surface EMG to better represent the effects of spike timing rather than action potential shape. In response, Christou and Neto (2010a, 2010b) pointed out that the mean discharge rate of MUs is better identified from interference EMG rather than rectified EMG (Farina et al., 2004), and that the frequency contribution of action potential shape to surface EMG is at frequencies outside those typically considered in corticomuscular coherence analysis. Certainly, rectification can assist in extracting multi-unit spiking activity from surface EMG signals, however, questions surrounding the technical validity of the method still remain. In several recent studies, the physiologically relevant/useful component of surface EMG (multi-unit spiking) was extracted by high-pass filtering the surface EMG signal prior to rectification,
thus removing many of the low frequency artifacts related to movement, activity in
adjacent muscles, and filtering effects of soft tissue (Potvin and Brown, 2004; Riley et al.,
2008; Brown et al., 2009). The recent controversies surrounding rectification indicate that
EMG filtering/processing may be an important consideration worth future investigation.
Recently, Negro and Farina, (2011a) showed the efficacy of identifying corticomuscular
coherence using only MU action potentials. Instead of filtering/rectifying the continuous
EMG signal, they identified the action potentials of individual MUs and processed their
activities into binary spike trains (as described in the previous section). The binary spike
trains were subsequently added together to form a ‘composite spike train’ for use in EEG-
EMG coherence analysis. The success of this method confirms that high frequency EMG
activity (i.e., MU spikes) contains sufficient information for identifying corticomuscular
coherence, and may be a powerful tool to use when a clear multi-unit signal can be
obtained.

The final step in identifying corticomuscular coherence is to conduct a coherence
analysis. This can be done exactly as outlined for two spike trains, or in a few cases,
time-frequency representations of the signals are used in order to track the temporal
progression of coherence with respect to a certain task (Kilner et al., 2000; Boonstra et al.,
2009). Figure 4 displays an illustrative example of an EEG-EMG coherence analysis. In
this example, coherence was calculated between EEG and rectified multi-unit activity
recorded from the human genioglossus muscle during voluntary tongue protrusion. The
coherence peak at about 20 Hz indicates significant corticomuscular coupling at this
frequency.
Figure 4: Example EEG-EMG coherence analysis. The top panel shows the raw EEG and EMG signals (top two traces) as well as the rectified EMG, calculated as the root-mean-squared (RMS) signal within a 10 ms moving window. The EEG signal was band-pass filtered between 1.5 and 150 Hz, and coherence was calculated between it and the RMS rectified EMG signal. The resulting coherence profile is shown below. The dashed line represents the 95% confidence level for this recording. Figure modified from Laine et al. 2011.

iii. Experimental findings
Corticomuscular coherence is consistently found in the beta (15-30) and gamma (30-50) frequency ranges (Conway et al., 1995; Salenius et al., 1997; Kilner et al., 2000; Baker and Baker, 2003; Yang et al., 2009). Both the strength and frequency of corticomuscular coherence appear to be task dependent. For example, increased contraction force is associated with an increase in beta band corticomuscular coherence, and larger forces are associated with the presence of gamma band coherence (Brown et al., 1998; Witte et al., 2007; Chakarov et al., 2009). Interestingly, the degree of gamma band corticomuscular coherence is not tightly associated with force level and for this reason is hypothesized to originate from processes involving attention/sensorimotor integration at the cortical level (Brown et al., 1998; Omlor et al., 2007; Chakarov et al., 2009). Similarly, Kristeva-Feige et al., (2002) found that beta band corticomuscular coherence could be greatly diminished when a subject’s attention to a motor task was divided by doing mental math, and that the frequency of corticomuscular coherence increased as the subjects completed motor tasks requiring higher levels of physical precision. An important distinction between beta and higher gamma frequency ranges is their association with movement. While beta band coherence is diminished by movement, gamma band coherence is not (Brown et al., 1998; Kilner et al., 1999; Marsden et al., 2000). Further, studies of a deafferented patient showed that during an isometric contraction task where force is modulated dynamically, gamma range corticomuscular coherence was absent while beta band corticomuscular coherence remained (Patino et al., 2008). This suggests that proprioceptive feedback may be unnecessary for beta band corticomuscular coherence, but may play a significant role in the development of gamma
band coherence, at least during a task that requires continual coordination of motor output with incoming feedback.

Fatigue is known to influence corticomuscular coherence in the beta frequency range during sustained submaximal isometric muscle contractions (Tecchio et al., 2006; Yang et al., 2009; Ushiyama et al., 2011). The effects of fatigue vary as a function of the motor pool being analyzed. Thus, fatigue decreases beta range corticomuscular coherence when measuring from the biceps brachii and brachioradialis (Yang et al., 2009), but increases coherence in recordings from the Tibialis Anterior muscle (Ushiyama et al., 2011) and extensor communis digitorum (Tecchio et al., 2006).

In the study of Yang et al., (2009), fatigue weakened corticomuscular coherence in the beta band even though both EEG and EMG amplitude increased. The dissociation between signal amplitude and signal coupling were further demonstrated by Baker and Baker, (2002), who recorded comparable beta band corticomuscular coherence before and after doubling cortical EEG power in that frequency range secondary to administration of diazepam. These results imply that amplitude relationships may not be a relevant component of corticomuscular coupling, at least in terms of coherence analysis.

Finally, corticomuscular coherence has a substantial dependency on central nervous system maturity. James et al., (2008) showed that beta/gamma band corticomuscular coherence (to the dominant forearm extensor muscle) is nearly absent in subjects under 12 years of age, is weak in subjects from ages 12-17, and only becomes consistently detectable in subjects 19-59 years of age.
In summary, both the strength and frequency of corticomuscular coherence can be manipulated by task and must be interpreted with respect to the anatomy of descending and ascending control of individual motor pools, along with subject-specific parameters such as age and state of attention.

**Neural control of the tongue**

The degree to which an individual source of input entrains MU activities is partly determined by the extent to which inputs are distributed (or shared) among MUs (Lemon, 1993) the strength of the signal from a given area, and the amount of noise introduced by other sources of input (Negro and Farina, 2011a), including afferent feedback. In some instances, similar tasks have completely opposite effects on synchronization among MUs of different muscles (Tecchio et al., 2006; Yang et al., 2009; Ushiyama et al., 2011). This underscores the need to interpret MU synchronization measures in the context of MU pool under investigation. For this thesis, attention is focused on control of hypoglossal motoneurons, particularly those which innervate the genioglossus muscle. What follows is an overview of the anatomical organization of hypoglossal motor control as well as the general activities of genioglossus (GG) MUs.

i. Anatomical organization of the HMN
Movement of the human tongue is controlled by the hypoglossal nerve (CN XII), whose cell bodies lie in the hypoglossal motor nucleus of the caudal medulla. Eight interdigitated muscles play a role in tongue movement. Since these muscles do not move bones around a joint, their activities are never truly functionally independent of each other and must be well coordinated. In fact, the tongue is considered a ‘muscular hydrostat’, much like an elephant’s trunk or the tentacles of a squid (Kier and Smith, 1985; Napadow et al., 1999). Tongue muscles are categorized as either intrinsic or extrinsic. Intrinsic tongue muscles change the shape of the tongue and include the vertical, transverse, superior and inferior longitudinal muscles. Extrinsic muscles originate on bone/cartilage and insert into the base of the tongue, allowing them to change the position of the tongue in the mouth. Of the extrinsic muscles, the styloglossus and hyoglossus retract the tongue whereas the genioglossus contributes to tongue protrusion along with the geniohyoid muscle, although the latter is not generally considered a lingual muscle (Chibuzo and Cummings, 1982). The remaining extrinsic tongue muscle, the palatoglossus, may be more appropriately classified as a pharyngeal constrictor muscle (Lowe, 1980), and is innervated by CN X.

The motoneurons of the tongue muscles are organized myotopically within the HMN. The nucleus itself consists of a dorsal and ventral subdivision, wherein axons from the dorsal subdivision form the lateral branch of CN XII that innervates the muscles that retract the tongue and axons from the ventral subdivision form the medial branch of CN XII that innervates the muscles that protrude the tongue (Barnard, 1940; Aldes, 1995). Within each subdivision, the MNs of extrinsic tongue muscles lie laterally to the MNs of
intrinsic tongue muscles, and despite some variation in this myotopic organization along the rostral-caudal axis (representing tongue topography in the base-tip direction), the MNs of the genioglossus are consistently localized to the ventrolateral portion of the HMN (Uemura-Sumi et al., 1988; Aldes, 1995). Axons of GG MNs primarily innervate the ipsilateral belly of the muscle, which can be functionally divided into a right and left halves despite a lack of clear visual evidence of such division (Snell, 1980). The number of hypoglossal MNs is quite large (~ 8000-9000 per side as a conservative estimate (Atsumi and Miyatake, 1987), and the average ratio of hypoglossal MNs to muscle fibers is thought to be particularly small (Sutlive et al., 1999, 2000).

Hypoglossal MNs are classified into two types based on their dendritic projections. The first type are the so-called ‘internal cells’ which can project dendrites to areas within the ipsilateral HMN and also across the midline, to the contralateral HMN (Wan et al., 1982), while the second type are called ‘external cells’ and project dendrites to bilateral HMN targets, as well as 1) the reticular formation, 2) the solitary nucleus and tract 3) the medial longitudinal fasciculus, and 4) the nucleus raphe obscurus (Wan et al., 1982; Altschuler et al., 1994). Wan et al (1982) suggest that projections into the medial longitudinal fasciculus and nucleus raphe obscurus may be major routes of cerebellar influence on the HMN, and it is clear that dendritic projections to the solitary nucleus give hypoglossal MNs access to a wide variety of visceral afferent input. The nucleus raphe obscurus may also be relevant in that the area is known to influence inspiratory and expiratory drive (Lalley et al., 1994, 1997; Peever et al., 2001). A final (less numerous) group of cells found in the HMN are not MNs, and can be classified as GABAergic
inhibitory interneurons which may play a role similar to Renshaw cells of the spinal cord (Sumi, 1969; Takasu et al., 1987; Takasu and Hashimoto, 1988), although this has not been functionally confirmed. These cells are small and mainly confined to the lateral portion of the HMN, where the MNs of extrinsic muscles are located (Boone and Aldes, 1984).

ii. Sources of synaptic input to the HMN

The HMN receives afferent information via projections from all divisions of the sensory trigeminal complex. The majority of this input is relayed from the mandibular branch of the trigeminal nerve (Marfurt, 1981; Jacquin et al., 1983; Shigenaga et al., 1988), the lingual branch of which conveys mechanoreceptive primary afferent information from the tongue (Jacquin et al., 1983). The sensory information relayed to the HMN from the trigeminal complex can be direct or indirect via the reticular formation (Pinganaud et al., 1999; Zerari-Mailly et al., 2001). Afferent input also exhibits species specificity. For example, spindles have been identified in both intrinsic and extrinsic tongue muscles (including genioglossus) of monkeys and humans (Cooper, 1953; Bowman, 1968; Kubota et al., 1975), while spindles appear to be absent from extrinsic tongue muscles of non-primate mammals suggesting that primates poses a unique motor control system for lingual movement (but see Smith, 1989). Finally, axons from the solitary nucleus project directly to the ipsilateral HMN (Norgren, 1978; Borke et al., 1983; Travers and Norgren, 1983), presumably transferring afferent feedback received through the glossopharyngeal and vagus nerves (Torvik, 1956; Contreras et al., 1982; Hamilton
and Norgren, 1984). Of course, glossopharyngeal and vagal feedback might also synapse directly onto the external dendrites of hypoglossal MNs within the solitary nucleus (Wan et al., 1982).

In addition to afferent/sensory input, there is evidence for direct and indirect (via reticular formation interneurons) projections from the primary motor cortex to the HMN (Holstege and Kuypers, 1977; Holstege et al., 1977; Snell, 1980; Alipour et al., 2002). Cortical projections to the HMN are bilateral for all muscles with the exception of the genioglossus, which is innervated mainly by the contralateral motor cortex (Snell, 1984). Once again, there is species specificity in the degree of direct vs. indirect cortical drive to the HMN. For example, in rats (Walberg, 1957; Travers and Norgren, 1983) and even some species of primate (Alipour et al., 2002) there are no direct projections from cortex to the HMN.

Hypoglossal MNs also receive inputs from a number of brainstem locations via neurons of the reticular formation, including respiratory-related input from the parafacial respiratory group (pons) and preBotzinger complex (medulla) and gustatory drives related to mastication, licking, and swallowing (reviewed in Sawczuk and Mosier, 2001). Interneurons of the reticular formation can be glutamatergic (Funk et al., 1993, 1997) cholinergic (Volgin et al., 2008) or glycine/GABAergic (Li et al., 1997). In addition, glutamatergic neurons of the kolliker-fuse nucleus (part of the pontine respiratory group) innervate hypoglossal MNs, and specifically those that innervate the genioglossus (Yokota et al., 2011). Especially in the case of respiratory-related inputs to the HMN, the
pathways by which this information is processed and delivered to motoneurons remains an open question (Fregosi, 2011).

iii. Activities of human genioglossus motor units

Single motor unit activity and whole muscle EMG recordings from identified tongue muscles provide a convenient, minimally invasive means of recording the activities of hypoglossal MNs. In the studies documented in this thesis, MNs innervating the genioglossus muscle are the focus of investigation. The genioglossus muscle is considered an ideal target of investigation in that it is easily accessible for purposes of recording, and because its MU activities are modulated by respiratory input and inputs arising in the motor cortex. The muscle is also of interest from a clinical perspective in that its dysfunction during sleep is linked to obstructive sleep apnea (Mezzanotte et al., 1992; Fogel et al., 2001; Saboisky et al., 2007).

For the most part, studies measuring motor unit or EMG activity from the GG have focused on the role of the GG in the maintenance of airway patency, which is accomplished through co-activation of protruder and retractor muscles (Fregosi and Fuller, 1997; Fuller et al., 1998). Historically, MUs of the GG have been classified by their activity relative to the respiratory cycle e.g., phasic inspiratory, phasic expiratory, tonic etc. (Tsuiki et al., 2000; Saboisky et al., 2006; Wilkinson et al., 2008, 2010). However, these activity patterns are not fixed properties of the MNs per se, and depend on the level/type of input drive they receive (e.g. Hwang et al., 1983; Lee and Fuller, 2010).
In terms of MU utilization within the HMN, a primarily recruitment-based activation profile has been observed when the muscle is primarily driven by (involuntary) respiratory-related drive (Nicholas et al., 2010; Richardson and Bailey, 2010; Saboisky et al., 2010). This may be due to the low levels of muscle force (<~25 % MVC) required to maintain airway patency during exercise, airway obstruction, or hypercapnia (Williams et al., 2000; McGinley et al., 2008; Richardson and Bailey, 2010). On the other hand, rate coding, comparable with what has been shown in spinal MN pools (e.g. Monster and Chan, 1977) is a dominant feature of GG MU activation produced by even modest levels of voluntary tongue protrusion (Bailey et al., 2007; Pittman and Bailey, 2009). These results suggest that MU utilization within the HMN varies depending on input (i.e., brainstem-mediated respiratory drive vs. cortical control). Direct investigation of this issue has not received much attention, especially given the relative dearth of information concerning voluntary control of GG MU activity.

iv. Missing information

To date, there have been no studies that assess the coordination between active hypoglossal MNs. Studies of MU-MU synchronization or of EEG-EMG coherence have, for decades, represented a major aspect of motor control research but have not been attempted within the context of lingual motor control at all under, any experimental conditions. This type of investigation can provide fundamental insights into the functional consequences of different sources of neural drive that impinge upon hypoglossal MNs. The simultaneous/overlapping delivery of synaptic input from
multiple sources (e.g. respiratory, cortical, afferent, etc.) and the complex anatomical distribution of these inputs onto the HMN make it unclear exactly how this information is integrated into a coordinated output. Measures of synchrony/coordination between MNs within a pool provide a framework within which to assess the role that a given input plays in determining motor output, and the extent to which it is able to influence MN activities in the face of competition from other sources of neural drive.

The studies documented herein are the first of their kind and provide much-needed new information regarding 1) the time scale and time course of common input to human hypoglossal MNs during rest breathing and voluntary tongue protrusion 2) respiratory-related synchronization between hypoglossal MNs as compared with other respiratory muscles in anesthetized rats, and 3) cortical entrainment of human hypoglossal MNs at both the population and single MU level during voluntary tongue protrusion.
PRESENT STUDY

The three manuscripts appended to this document appear in the Journal of Neurophysiology (Laine and Bailey, 2011; Laine et al., 2011; Rice et al., 2011). Each study is a novel contribution to our understanding of the effects of synaptic input on synchrony between hypoglossal MNs or between hypoglossal MNs and a particular source of input. Details regarding methods, results, and conclusions of the studies are provided in each manuscript. What follows is a brief summary of the major findings and avenues for future investigation.

Study 1: Common Synaptic input to the human hypoglossal motor nucleus

In this study, single MU activities were recorded from the right and left bellies of the genioglossus muscle of human subjects engaged in either rest breathing or voluntary tongue protrusion for periods of about 2 minutes. In each session, two single MUs were recorded simultaneously, either from the same or opposite bellies of the genioglossus muscle. The activities of each MU pair were analyzed for the presence of 1) short-term synchrony, 2) common drive, and 3) coherence. Since genioglossal MNs on each side of the medulla primarily innervate ipsilateral muscle fibers, we were able to determine if synchronous MU activity stemmed from bilaterally or unilaterally distributed sources of input to the HMN. In evaluating motor unit synchrony during rest breathing, we gauged the effects of involuntary, brainstem-mediated inputs (e.g. respiratory drive). In the tongue protrusion condition, we assessed the effects of voluntary input arising in motor cortex.
We found that in rest breathing, MU pairs recorded from the same or opposite bellies of the GG showed no short-term synchronization with each other, indicating weak or modest input via shared axonal branches from premotor sources of involuntary (respiratory-related) input. In contrast, in tongue protrusion, we saw significant levels of short-term synchrony in unilaterally recorded MU pairs, consistent with unilaterally distributed branched axons arising from cortical premotor neurons (Snell, 1980). While bilaterally recorded MUs did not show statistically significant levels of short-term synchrony, there was significantly more simultaneous spiking in bilaterally recorded MUs during tongue protrusion than in rest breathing. Accordingly we concluded that cortical drives also exert a weak bilateral effect on hypoglossal MNs. We found evidence of a moderate degree of common drive within and between GG bellies during in both conditions; although common drive was greatest in unilaterally recorded MU pairs during tongue protrusion. This finding also supports a primarily unilateral distribution of common input from the motor cortex. Low frequency coherence showed the same general trend as common drive, and no recording scheme or task condition evoked coherence > ~ 10 Hz. The latter result was surprising given cortical drive to MN pools is known to contain 15-40 Hz oscillatory component capable of synchronizing MN activities (Farmer et al., 1993; Conway et al., 1995; Salenius et al., 1997; Brown et al., 1998).

Study 2: Synchronization of presynaptic input to motor units of tongue, inspiratory intercostal, and diaphragm muscles

In this study, pairs of MUs were simultaneously recorded from muscles of the tongue (genioglossus and hyoglossus), and chest-wall (diaphragm and external intercostals) of
anesthetized, spontaneously breathing rats. In this case the objective was to determine the effects of respiration-related presynaptic inputs on MU synchrony within each muscle, and to determine if differences existed between respiratory synchronization of hypoglossal MNs in comparison with those innervating chest wall muscles. In addition, because the genioglossus and hyoglossus exert opposing effects on the tongue but are coactivated during inspiration (Fregosi and Fuller, 1997; Fuller et al., 1998), we examined synchronization across tongue muscles. In these studies, coherence analysis was used to quantify MU-MU synchrony.

We found that all muscles studied showed MU-MU coherence up to about 8 Hz, mainly reflecting the frequency of breathing and its harmonics (since each MU was active for only a portion of the respiratory cycle). This respiratory drive also was present in the coherence profiles of MU pairs recorded from different tongue muscles. Interestingly, MU pairs recorded from chest-wall muscles showed far stronger coherence in the 10-20 Hz range than MU pairs of the tongue muscles. We concluded that respiratory-related drive likely contains high frequency components, but that these components only evoke MU-MU synchronization in the chest-wall. We suggest that diversity of presynaptic inputs converging onto the hypoglossal motor nucleus may interfere with the ability of high frequency respiratory drive to evoke synchrony in this MN pool.

**Study 3: Cortical entrainment of human hypoglossal motor unit activities**

In Study 3, we recorded multi-unit EMG (i.e., whole muscle) or single MU activities simultaneously with EEG in human subjects performing a simple tongue protrusion. In this case our objective was to assess the effects of cortical drive on the synchrony of human hypoglossal
motoneuron activities during a voluntary task. The results of the previous studies (Study 1 and 2) indicated that hypoglossal MUs were not strongly synchronized at frequencies greater than ~ 10 Hz. Importantly, the latter finding is at odds with previous studies in limb muscles show that cortical oscillatory drive in the 15-40 Hz frequency range synchronizes MU activities (Farmer et al., 1993; Conway et al., 1995; Salenius et al., 1997; Brown et al., 1998). In view of this discrepancy and in light of in vitro studies that indicate the efficiency of an input in driving MU activity relates to input frequency (Hunter et al., 1998; Funk and Parkis, 2002; Parkis et al., 2003; van Brederode and Berger, 2008), we sought to investigate cortical coupling with GG activity more directly. In this case we utilized standard EMG-EEG coherence analysis and devised a novel technique that permitted us to track the effects of fluctuations in EEG on the timing of single MU action potentials (i.e., EEG phase-locking with single MU activity).

We found that cortical oscillatory input in the 15-40 Hz frequency range does synchronize hypoglossal motoneuron activities, however, this effect was found to be weak and inconsistent across trials. In contrast, we show that EEG phase-locking with single MU activity is a robust feature of corticomuscular coupling. We were also able to track the influence of 15-40 Hz cortical drive on the timing of single MU activities over the course of single recordings. We found that phase-locking of single MUs to EEG oscillations is a time-varying effect, that is, brief and episodic in nature. We suggest that this is due to the influence of time-varying sources of synaptic noise, and may explain the lack of 15-40 Hz MU-MU coherence found in Study 1.
Future directions

An important theme in this thesis is that sources of neural drive influence the degree, temporal progression, and timescale of MU synchronization. Physiologically, different types of drive (e.g. respiratory and voluntary) impinge on MNs simultaneously. The studies outlined above primarily focused on isolating or comparing the effects of voluntary and respiratory drives on MU synchronization. With this foundation in place it is now possible to study the integration of these drives over time, which could yield new insights into lingual muscle control, or motor control in any system.

i. Respiratory-related activities

In Study 3, we showed that single MU phase-locking with EEG occurred periodically over time during static tongue protrusion. A simple extension of this work would be to evaluate time-varying synchronization with respect to the respiratory cycle. Thus, if the overlap of respiratory and cortical drives decorrelates HMN activity and beta/gamma band input from the primary motor cortex, we might predict a higher degree of corticomuscular coupling during the phase of respiration with the least overall MU activity. By extension, all three indices of synchronization applied in Study 1 (short-term synchrony, common drive, coherence) could be modified to permit investigation of respiratory-phase related changes in MU-MU synchronization during rest breathing and during tongue protrusion. Understanding any respiratory-related influences on MU-MU coordination, or corticomuscular coupling, is a necessary first step toward understanding fundamental aspects of oromotor control. Towards this end, respiration-related effects on MU activities could be studied under conditions where respiration is controlled
voluntarily (cortical) or automatically (i.e. central pattern generator mediated) could yield novel insights into the delivery of respiratory-related efferent or afferent drives to the HMN. For example, in human subjects it is possible to alter breathing frequency and/or volume either via exercise or by manipulation of inspired gas content in order to measure MU coordination during different levels of involuntary respiratory drive. If the breathing rates/volumes are then displayed to the subject, it would be possible for them to volitionally modulate their breathing to match the pattern induced by exercise or gas-manipulation. In this manner we could compare the effects of voluntary vs. involuntary control of respiratory drives on MU synchrony. As outlined in earlier, the HMN processes a wealth of afferent input relayed via the solitary nucleus and sensory trigeminal complex. This input provides additional potential for experimental manipulation. For example, airway resistance can be manipulated secondary to manipulations through inhaled gas content. In addition, it might be possible to apply vibratory stimuli to the chest or throat and assess MU synchronization by such rhythmic afferent input. Because vibration of the throat occurs with vocalization, such an investigation might prove a useful first step toward eventual speech-related analyses.

ii. Voluntary tongue movement

Several decades of work using synchronization measures to characterize MU coordination during a range of motor tasks can now be applied to the study of oromotor control. Such investigation could greatly improve our understanding of how different sources of neural drive gain influence over MNs, especially those of the tongue. For
example, MU-MU coordination and corticomuscular coherence have not been studied during tongue motion or as a function of variations in tongue muscle force, fatigue, or during speech or speech-like movements. There are also no investigations of the effects of age or attention on measures of synchrony in this motoneuron pool. If we consider the functions of the human tongue, it would be reasonable to focus on dynamic (even ballistic) movements rather than constant position/force experiments used previously in evaluations of synchrony in spinal motoneuron pools. The simple protrusions attempted in studies 1 and 3 are an ideal basis on which to build (and against which to compare) the results of more complex motor tasks. The analysis of movement tasks will entail time domain or time-frequency domain measures of MU synchronization, and to that end, the methods utilized in study 3 may be of particular relevance. Given that cortical effects on short-term synchronization appeared to be unilaterally reflected in the GG (see study 1), it may be that cortical drive entrains MN activities only in contralateral HMN. We could further predict that cortical entrainment of MN activities would be similar bilaterally during simple tongue protrusion, but potentially show differences during speech production, which is considered to be lateralized to the left hemisphere (Simonyan et al., 2009; Sowman et al., 2009). Such studies may help to bridge the gap between speech-related research and studies of oromotor control.

The phenomenon of MU phase-locking with EEG activity was characterized for the first time in any muscle in Study 3. A logical extension of this work would be to compare the degree of phase-locking observed in hypoglossal MNs with more commonly studied muscles, such as the FDI. The fact that single MUs phase-locked to EEG for
short time periods suggested that GG MUs may not synchronize with EEG oscillations at the same time as each other, which could explain the lack of MU-MU coherence observed in Study 1 and the weak EEG-EMG coherence found in Study 3. This hypothesis could be tested simply by recording two single MUs from a belly of the GG while EEG is recorded over the contralateral motor cortex. Subsequently, the time course of single MU phase-locking with EEG could be assessed with respect to the timing of simultaneous discharges between the two MUs.

iii. Patient populations and clinical interventions

The measures outlined in this thesis represent quantifiable indices of neural communication to motor pools. As such, they may be of use in characterizing normal and abnormal states, and tracking alterations in motor control induced by disease processes. These same measures may also provide means of assaying the effect/s of clinical intervention, or providing information to brain-machine interfaces, where intended movements are first identified and then translated into the control of an external device.

To date, hypoglossal motor unit synchrony and corticomuscular coupling has not been assessed in patient populations, as they have for motor units of the limbs. Such investigations could yield important information concerning the transmission of rhythms to motor pools in conditions such as Parkinson’s disease and tremor, or the transmission of normal input to a pathologically reduced motor pool, as in ALS. Investigation of corticomuscular coupling may also be of relevance and utility for understanding the nature and effects of disorganized premotor input to the HMN, as may be the case in
individuals who stutter. With respect to respiratory-related input to the HMN, individuals with obstructive apneas or hypopneas are ideal populations for investigating the extent to which brainstem CPG input synchronizes hypoglossal motoneuron activities when their final output known to be dysfunctional.

Based on the findings presented in this dissertation, the relative excitability and coordination of hypoglossal motoneuron activities depends partly on the frequency and timing of inputs. This suggests avenues for manipulation of motoneuron excitability and coordination. For example, afferent stimulation (e.g. vibration of the chest wall or throat) delivered at the ‘preferred’ frequency of hypoglossal motoneurons, may have an impact on cortical or respiratory-related activation of these motoneurons. As our understanding of the functional consequences of overlap between cortical and non-cortical inputs to the HMN becomes clear, it may be possible to reduce the impact of non-cortical sources of input in order to improve voluntary muscle control, or to reduce the impact of aberrant rhythmic drive to reduce tremor.

In Study 3, we showed that corticomuscular coupling can be tracked over time, using the activity of a single motor unit. Such an approach could be used to track any change/s in the strength of cortical inputs (e.g. after stroke) as pharmacological or physical therapies progress. In cases where there is reduced connectivity between the cortex and motoneurons, indices of coupling between single motor units and EEG may be a more sensitive means of identifying corticomuscular coupling than commonly-used measures which quantify cortical entrainment of whole-muscle activity. Along the same lines, it might be possible to devise a device that can stimulate muscle contraction (or
control a prosthesis) in periods of heightened synchrony between a few remaining (albeit functionally useless) motor units and cortical EEG.

Clearly, there are a great many possibilities for future experiments involving coordination of MUs in the HMN. The work presented herein will ideally provide a foundation for such efforts, and hopefully brings a new set of analytical methods to the study of oromotor control that have previously been reserved for other muscle systems.
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APPENDIX A: COMMON SYNAPTIC INPUT TO THE HUMAN HYPOGLOSSAL MOTOR NUCLEUS.

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Running head: Human hypoglossal motor neurons.
Abstract

The tongue plays a key role in various volitional and automatic functions such as swallowing, maintenance of airway patency, and speech. Precisely how hypoglossal motor neurons, which control the tongue, receive and process their often concurrent input drives is a subject of ongoing research. We investigated common synaptic input to the hypoglossal motor nucleus by measuring the coordination of spike timing, firing rate, and oscillatory activity across motor units recorded from unilateral (i.e. within a belly) or bilateral (i.e., across both bellies) locations within the genioglossus (GG), the primary protruder muscle of the tongue. Simultaneously-recorded pairs of motor units were obtained from 14 healthy adult volunteers using tungsten microelectrodes inserted percutaneously into the GG while the subjects were engaged in volitional tongue protrusion or rest breathing. Bilateral motor unit pairs showed concurrent low frequency alterations in firing rate (common drive) with no significant difference between tasks. Unilateral motor unit pairs showed significantly stronger common drive in the protrusion task compared to rest breathing, as well as higher indices of synchronous spiking (short-term synchrony). Common oscillatory input was assessed using coherence analysis, and was observed in all conditions for frequencies up to ~5 Hz. Coherence at frequencies up to ~10 Hz was strongest in motor unit pairs recorded from the same GG belly in tongue protrusion. Taken together, our results suggest that cortical drive increases motor unit coordination within but not across GG bellies, while input drive during rest breathing is distributed uniformly to both bellies of the muscle.
**Introduction**

The tongue is a unique structure both with respect to its composition and its function. Its muscles are innervated by the hypoglossal nerve (cranial nerve XII) whose cell bodies are located in the hypoglossal motor nucleus (HMN) of the caudal medulla. The HMN receives input from multiple sources, including the primary motor cortex and several brainstem central pattern generators related to critical functions such as chewing, swallowing and respiration (reviewed in Sawczuk and Mosier 2001). Neurophysiological studies of the tongue have typically focused on its primary protruder muscle, the genioglossus (GG). The GG is composed of a right and left belly, each controlled by the ipsilateral HMN. Within each HMN, GG motor neurons receive projections from the contralateral primary motor cortex (Snell 1980) along with bilaterally distributed drive from respiration-related premotor neurons (Ezure and Tanaka 2006; Peever and Duffin 2001; Tarras-Wahlberg and Rekling 2009).

The effect that synaptic input has upon the output of a motor neuron pool (i.e., firing rates, burst patterns) varies as a function of the source of the input and its distribution onto the motor nucleus. Of particular interest is how different inputs form and organize groups of motor neurons in the pool to subserve a particular behavior. One means of assessing this *in vivo* is to examine the correlated activities of simultaneously active single motor units under a range of conditions (e.g. Datta and Stephens 1990; De Luca et al. 1982; De Luca and Erim 1994; Farmer et al. 1993; Rosenberg et al. 1989; Sears and Stagg 1976). Thus, common fluctuations in firing rates and synchronous
spiking among a population of motor neurons can be used to characterize the organization of different sources of synaptic input.

In the present study, we sought to characterize the strength and temporal patterns of correlated motor unit activity within and across bellies of the GG in rest breathing and voluntary tongue protrusion. This allowed us to compare automatic, brain-stem mediated control with voluntary control from the cortex. We predicted that in the rest breathing condition, we would measure a high degree of correlated activity in motor units recorded both from within and across bellies of the GG, since respiratory drive to the HMN is bilaterally distributed. Conversely, because cortical premotor neurons that control the GG project to the contralateral HMN we predicted that tongue protrusion would increase correlated activity between motor units within but not across bellies of the GG.

Methods

Recordings were obtained from 14 (8 male, 6 female) healthy adult volunteers (mean body mass index (BMI) = 21.9 ± 2.3 (SD), mean age = 24.4±3.5 (SD) years). All procedures were approved by the Human Subjects Committee at the University of Arizona, and all subjects gave informed consent prior to participation.

General procedures

For each subject, the depth to the inferior border of the GG muscle was determined using ultrasonography (Pro Sound 3500, Aloka, Tokyo, Japan) (Eastwood et al. 2003). Paired single motor unit recordings were obtained in rest breathing or
volitional tongue protrusion. Each motor unit pair was recorded under one experimental condition. For rest breathing, subjects lay supine in a dental chair and were given no instructions other than to rest quietly with their eyes open. No audio or visual feedback was provided in this task. For volitional tongue protrusion, subjects sat upright in the dental chair and were instructed to slowly move the tongue forward toward the teeth until motor units were recruited. In this task, subjects were provided audio and/or visual feedback from one of the recorded units and asked to maintain a steady firing rate. In both tasks recordings were sustained for ~2 minutes (126 ± 55 s).

**EMG recordings**

Single motor unit action potentials were recorded using tungsten needle electrodes (100 KΩ at 1 KHz, 1-5 μM tip diam., 250 μM shaft diam., Frederick Haer, Bowdoinham, ME) inserted percutaneously into the GG, and referenced to surface electrodes on the mastoid process. A ground electrode was positioned on the right clavicle. Pairs of simultaneously recorded single motor units were obtained from one side of the GG (unilateral placement) or opposite sides of the GG (bilateral placement). Electrodes were positioned ~1.0 cm from the midline and ~2.0 cm from the other electrode. EMG signals were sampled at 33 KHz, amplified (20,000 x), and band pass filtered from 0.3-3 KHz (Model 15 Grass Instruments, West Warwick, RI). The signals were acquired and stored using a Cambridge Electronic Design 1401 interface and Spike2 software (Cambridge Electronic Design, Cambridge UK).
Data analysis

Single motor unit action potentials were discriminated offline in Spike2, using a template-matching algorithm based on waveform shape and amplitude. The results were checked by visual inspection and, where necessary, corrected manually. Indices of short-term synchrony and common drive were obtained for motor unit pairs using custom Spike2 scripts, and coherence analysis was carried out in Matlab 7.04 (The MathWorks, Natick, MA, USA). For the purpose of comparison, the recordings were split into four groups according to each combination of electrode location (unilateral or bilateral) and task condition (protrusion or rest breathing). All statistical analyses were carried out in Matlab.

Details regarding the derivation and analysis of each measure of motor unit correlation are provided below:

Common Drive Index (CDI)

Concurrent fluctuations in the firing rates of motor units across a pool reflects a widespread common input drive which can be quantified using the method described by De Luca et al. (1982); herein referred to as the common drive index (CDI). Briefly, motor unit spike times were used to construct binary impulse trains (1 ms resolution), which were then convolved with a 400 ms Hann window and high-pass filtered (0.75 Hz) using a 3rd order Butterworth filter. The resulting smoothed firing rate traces were used to construct a cross-correlogram from each motor unit pair, with the largest correlation coefficient within ±100 ms of zero lag taken as the CDI. The CDI is therefore a number
between -1 and 1, with 1 indicating perfect correlation and 0 representing no correlation between the spike firing rates. The key aspects of this calculation are presented in Figure 1 (Panels A-C). Panel A depicts a short time-span (10 s) of discriminated action potentials for a motor unit pair. Following conversion of spike trains to smoothed firing rate traces (Panel B), it is evident that the two motor units show common fluctuations in mean firing rate. The extent to which motor unit firing rates are similarly modulated by synaptic input is reflected in the cross-correlation of their smoothed firing rate traces, calculated over the entire recording duration (Panel C). The cross-correlogram shows a peak of 0.76, which is the CDI. CDI values were compared across experimental groups using a Kruskal-Wallis ANOVA with Tukey’s HSD post-hoc tests used for pairwise comparisons.

Short-term synchrony

Short-term synchrony refers to the tendency of single motor units to fire simultaneously more often than expected by chance. The increased probability of synchronous firing occurs when motor neurons share a physical connection to a common pre-synaptic source (Datta and Stephens 1990; Sears and Stagg 1976). We quantified the magnitude of short-term synchrony by calculating the K’ index, which is a ratio expressing the number of synchronous spikes relative to the number expected by chance (Ellaway and Murthy 1985). Panel D in Figure 1 demonstrates several aspects of the calculation. The spike times were used to construct a cross-correlation histogram (bottom of panel D, bin size = 1 ms, lags = ±100 ms). A peak-region (vertical dashed
lines) was identified using the cumulative sum derivative (Ellaway 1978) of the cross-correlation histogram (Panel D, top trace). The borders of the peak-region were identified as the points at which the cumulative sum derivative trace exceeded 10 and 90% of the difference between its maximum and minimum values (Schmied et al. 1993). Histogram bins falling within the peak-region are taken to represent synchronous spiking. The mean and standard deviation of the off-peak bin counts (i.e., the region outside the ±40ms range) were used to estimate the level of synchronous firing attributable to chance (Keen and Fuglevand 2004). The K' index was then calculated by dividing the average count in the peak-region by that of the off-peak region. The average spike count in the peak region was then compared to chance using the mean and standard deviation of off-peak bin counts (z-score). If the level of synchronous activity did not exceed chance (z-score <1.64, translating to a one-tailed p-value <0.05), K’ was re-calculated using a fixed peak-region of 11 ms centered at zero lag (Semmler and Nordstrom 1995). As with the common drive analysis, K’ values were compared across experimental groups using a Kruskal-Wallis ANOVA with Tukey’s HSD post-hoc tests used for pairwise comparisons.

**Coherence**

Different synaptic inputs impinging onto a MN pool vary in regard to their frequency composition. Coherence analysis is a frequency domain technique which can reveal distinct oscillatory drives to MN pools using pairs of simultaneously recorded motor units (e.g. Farmer et al. 1993; Rosenberg et al. 1989). This method produces a coefficient between 0 and 1 which describes the correlation between the spike trains at
each frequency. Here we converted raw spike times into impulse trains (sampling frequency = 1000 Hz), and coherence was calculated with Matlab’s mscohere function using un-weighted, non-overlapping data segments 2048 ms in length. This yielded coherence estimates at a frequency resolution of 0.49 Hz. The significance of coherence was assessed by determining the 95% confidence level for each motor unit pair according to the equation $1 - 0.05(1/(N-1))$, where N is the number of disjoint data segments used in the analysis (Carter 1987; Rosenberg et al. 1989). Figure 1E shows the coherence analysis of the same motor unit pair as shown in panel A. The horizontal line marks the 95% confidence limit. This motor unit pair shows significantly correlated activity at frequencies up to about 10 Hz.

Two methods were used to assess coherence across experimental conditions. First, the number of motor unit pairs exhibiting significant coherence at a given frequency was determined for each experimental group. Fisher’s exact test was then used to compare these numbers across groups. Second, we compared coherence magnitudes. To facilitate this type of comparison, coherence values (C) were normalized using Fisher’s transform ($z = \text{atanh}(\sqrt{C})$), and weighted according to the number of segments used in determining the initial coherence estimates. A two-tailed unequal variance t-test was then used to compare the transformed coherence values across groups at every frequency up to 500 Hz. The average within-group coherence for each frequency was calculated by re-transforming the weighted mean of Fisher’s z-scores.

**Results**
A total of 91 motor unit pairs were recorded. The average single motor unit firing rate was 18.8 ± 4.3 Hz. Table 1 shows the mean ± SD of CDI and K’ values grouped according to experimental condition. Also shown in Table 1 are the numbers of motor unit pairs recorded in each location and task.

*Common drive*

The results of the common drive analysis are summarized in Figure 2A. The box and whisker plots show the median (midline), inter-quartile range (box and whiskers), and outliers (‘+’ signs) of the CDI values calculated for motor unit pairs within each experimental condition. Common drive values did vary significantly across experimental groups (p <0.001, Kruskal-Wallis ANOVA). More specifically, motor unit pairs recorded unilaterally in the tongue protrusion task had substantially higher levels of common drive than the other experimental conditions, which were not significantly different from each other. The influence of volitional input on common drive seems to be reflected only in motor unit pairs located within the same belly of the GG.

*Short-term synchrony*

Figure 2B shows box-and-whisker plots summarizing the distribution of K’ values calculated from motor unit pairs in each experimental condition. No motor unit pairs showed significant short-term synchrony in the rest breathing task, whereas seven motor unit pairs showed significant synchronization in the tongue protrusion task. All seven pairs showing significant synchronization were recorded from unilateral sites and had peak widths <10 ms. K’ values generally were small but varied significantly across
groups (p=0.007, Kruskal-Wallis ANOVA). The effect of task on short-term synchrony became most apparent when recording location was ignored and motor unit pairs recorded in protrusion were compared to those recorded in rest breathing. Tongue protrusion was associated with larger K’ values, and although the magnitude of this difference was small, it was strongly significant (p<0.001, Kruskal-Wallis ANOVA). In fact, the K’ values in the protrusion task remained significantly larger than those of rest breathing even after all large K’ values (z-score >1.64) were excluded from the data set (p = 0.017, Kruskal-Wallis ANOVA). In contrast, there was no significant difference in K’ values across recording locations (unilateral vs. bilateral) when task was ignored (p = 0.205, Kruskal-Wallis ANOVA). To rule out any effects that firing rate may have had on these results, we calculated the geometric mean firing rate for each MU pair and found no difference across tasks (p = 0.06, t-test), and no correlation between firing rate and K’ values in general (Pearson’s rho = -0.17, p = 0.115). Importantly, recording durations were no different for each task (p = 0.096, t-test).

Comparing common drive and short-term synchrony across all recorded motor unit pairs, we found a significant correlation between K’ and CDI values (Pearson’s rho = 0.38, p <.001). In contrast to the short-term synchrony analysis, when CDI values were grouped by task (ignoring recording location), no significant effect of task was found (p = 0.14, Kruskal-Wallis ANOVA).

*Coherence*
Coherence was analyzed in two ways, first we assessed the proportion of motor unit pairs within each group that showed significant coherence, and second, we assessed coherence magnitudes directly.

The results of the first analysis are depicted in the top two panels of Figure 3, which show the proportion of motor unit pairs having significant coherence at each frequency. Panel A compares unilateral motor unit pairs recorded in tongue protrusion (black) and rest breathing (gray) and Panel C compares bilateral motor unit pairs recorded in tongue protrusion (black) and rest breathing (gray). The histograms in Panels A and C were tested for statistical differences at each frequency using Fisher’s exact test. The resulting p-values are plotted for each frequency below the coherence histograms. Since frequencies above about 50 Hz showed no relevant effects (in either type of analysis), we have displayed frequencies up to 50 Hz in Figure 3. There was evidence of significant coherence for frequencies <2.0 Hz in the majority of recordings. At higher frequencies (up to ~10 Hz), motor unit pairs recorded unilaterally during tongue protrusion showed significant coherence more often than any other group, all of which had similar coherence profiles to each other.

The outcome of the second analysis is shown in Panels B and D, which compare average coherence magnitudes as a function of recording location and condition. Again, results of the statistical comparison between the groups (t-tests) at each frequency are plotted at the bottom of Panels B and D. Similar to the proportion analysis, coherence at frequencies up to ~10 Hz was strongest on average in motor unit pairs recorded
unilaterally in the tongue protrusion condition. The average coherence profiles of all other groups were very similar to each other.

Discussion

We explored synaptic drives associated with voluntary and involuntary control of the human tongue. The influence of synaptic drive onto a motor neuron pool can be evaluated in terms of concurrent fluctuations in motor unit firing rates, simultaneous spiking, and correlated oscillatory activity. Accordingly, we measured common drive, short-term synchrony, and coherence between simultaneously recorded GG motor units in rest breathing and tongue protrusion.

Common Drive

Common drive has primarily been studied in hand muscles during isometric contractions (De Luca et al. 1982, 2009; De Luca and Mambrito 1987; Erim et al. 1999; Garland and Miles 1997; Kamen et al. 1992; Marsden et al. 1999; Negro et al. 2009; Sauvage et al. 2006; Semmler and Nordstrom 1995, 1998; Semmler et al. 1997), where typical CDI values fall between 0.4 and 0.6. Although the absolute level of force does not appear to affect common drive (De Luca et al. 1982; Erim et al. 1999), common fluctuations in mean firing rate are thought to underlie fluctuations in force output (De Luca et al. 1982; Reviewed in De Luca 1985). In general, the strength of common drive declines in tasks which require fine motor control, whereas gross motor activities are
associated with stronger common drive values. For example, common drive values for motor units of the first dorsal interosseus muscle are larger after cerebellar stroke (Sauvage et al. 2006), and reduced in skilled musicians (Semmler and Nordstrom 1998). Consistent with this general idea are findings that common drive is typically greater in muscles with lower spindle density, i.e., with less proprioceptive feedback (De Luca et al. 2009, Kamen et al. 1992), in fatigued muscles (Contessa et al. 2009), in trunk muscles when used for postural control (Mochizuki et al. 2006), and across synergist muscle/motor unit pools (De Luca and Mambrito 1987; Marsden et al. 1999).

In the present study, we were able to investigate levels of common drive in a muscle which fulfills a respiratory postural role (i.e., related to airway patency), and yet is subject to a high degree of volitional control by the motor cortex. Conveniently, we were able to probe the distribution of common drive to unilateral or bilateral hypoglossal nuclei since right and left sides of the GG are controlled from the ipsilateral HMN (Snell 1980). Our findings confirmed our initial prediction that in rest breathing, the magnitude of common drive would be of equal strength for motor unit pairs recorded within and across bellies of the GG. The actual CDI values were fairly strong, comparable with what the above-cited studies recorded for finger muscles under volitional control. Since cortical projections to GG motor units are primarily unilateral, we predicted that levels of common drive would be greater for unilateral versus bilateral recording locations. Our findings confirmed this prediction. Interestingly, during tongue protrusion, CDI values differed from those recorded during rest breathing only in unilaterally recorded motor units. The lack of any detectable effect of tongue protrusion
on CDI values measured from bilaterally recorded motor unit pairs could indicate that cortical premotor activity is not temporally correlated across hemispheres (at least within the frequency range measured by the CDI). An additional consideration is that cortical input to the two HMN may still be subject to slightly different local processing within each individual HMN, and that such processing might de-correlate the final motor outputs.

*Short-term synchrony*

The extent of short-term synchronization among motor neurons is affected by the density of their premotor inputs (Lemon 1993), as well as factors which modify the overall influence of those projections on the final output of the motor neuron pool. For example, factors such as task (Adams et al. 1989; Bremner et al. 1991) or attention (Schmied et al. 2000) can affect short-term synchrony. Like common drive, short-term synchrony has been found in synergist muscle/motor unit pairs (Powers et al. 1989), however, in studies where proprioceptive feedback or fatigue altered common drive, synchrony remained unchanged (Contessa et al. 2009; Garland and Miles 1997). Thus, while common drive and short-term synchrony can be correlated (Semmler et al. 1997), they do not appear to arise from the same source (Semmler et al. 1997; Negro et al. 2009). Our observations are in agreement with these previous findings, as CDI and K’ values were moderately correlated, but not necessarily predictive of one another.

We expected that in rest breathing, when the GG acts to maintain airway tone, we would observe similar levels of short-term synchrony in unilateral and bilateral motor
unit pairs. Our data confirmed this prediction; however, it is worth noting the complete absence of short-term synchrony in either unilateral or bilateral motor unit pairs. This indicates that, of the motor neurons that are active during rest breathing, few share premotor axon branches whose input is strong enough to evoke simultaneous spiking. The implication that individual motor neurons may respond weakly to involuntary drive is supported by previous findings that whole muscle EMG recorded from the GG reaches only ~20-25% of its maximum inducible level when respiratory drive is high, as in exercise (Williams et al. 2000), airway obstruction (McGinley et al. 2008), and hypercapnia (Richardson and Bailey 2010). Further, a relative weakness of respiratory drive compared with direct cortical input could explain why common modulation of firing rates, as measured by the common drive index and coherence, was larger in the protrusion condition compared with rest breathing.

All motor unit pairs that showed significant levels of short-term synchrony were recorded unilaterally during the protrusion task. The fact that significant levels of short-term synchrony were only observed in unilateral motor unit pairs appears to reflect the unilateral nature of cortical projections to the HMN (Haerer 1992; Snell 1980). Ignoring recording location, motor unit pairs had slightly larger K’ values in the tongue protrusion condition than the rest breathing condition, even when all significantly large K’ values were excluded from the data set. This implies a weak bilateral effect of cortical drive on short-term synchrony in addition to the stronger unilateral effect. Aside from shared axonal input, synchronization in the activity of premotor neurons can increase the probability of simultaneous spiking (i.e. higher K’ values) between motor neurons
(Kirkwood et al. 1982; McAuley et al. 1997; Murthey and Fetz 1996). We did not observe the characteristic broad-peaks or fast oscillations which are associated with premotor synchronization (Kirkwood et al. 1982). Another way in which cortical drive may have slightly increased synchronous firing in bilateral motor unit pairs arises from the anatomy of HMN motor neurons themselves. Motor neurons may extend their dendrites from one HMN to the other (Altschuler et al. 1994), and therefore receive cortical input projected onto the opposite HMN. If such connections were rare or weak, their influence on short-term synchrony could remain undetectable at the level of individual motor unit pairs while still influencing group comparisons.

**Coherence**

We used coherence to identify oscillatory drives descending upon the HMN pool (Farmer et al. 1993; Rosenberg et al. 1989). The physiological interpretation of coherence varies depending on frequency (for reviews see: Brown 2000; Grosse et al. 2002). For example, coherence in the range of 1 to 5 Hz describes the same phenomenon that is measured by the common drive index (Myers, 2004). Coherent activity at frequencies between 6 and 12 Hz may reflect a distinct type of drive (Erimaki and Christakos 1999, 2008) associated with movement (Kakuda et al. 1999; Vallbo and Wessberg 1993), feedback from muscle spindles (Erimaki and Christakos 2008), or force-tremor (Marsden et al. 2001). Finally, coherence in beta (15-30 Hz) and gamma (30-60 Hz) frequency bands is associated with short-term synchrony (Farmer et al. 1993,
We found significant coherence at frequencies under ~2 Hz in nearly all GG motor unit pairs recorded. The fact that coherence in this range did not require cortical drive is in agreement with previous findings that motor unit firing rates remain coherent at these (low) frequencies even following capsular stroke (Farmer et al. 1993). At higher frequencies covered by time-domain common drive measures (typically up to ~5 Hz), unilateral motor unit pairs recorded during tongue protrusion showed the strongest coherence, similar to the results obtained when using CDI as the measure of common firing rate modulation. The agreement of time domain (CDI) and frequency domain (coherence) measures of common firing rate modulation suggest that this is a robust feature of GG motor unit activity and one that is particularly responsive to cortical input. Also in the tongue protrusion task, there was increased coherence in the 6 to 10 Hz range. In our study, subjects did not perform dynamic tongue movements, but we can not rule out a possible association with tremor or feedback. Since activity in the 6-10 Hz range is well below the average firing rate of GG motor units (~20 Hz), coherence in this range may simply represent an extension of lower frequency common drive rather than a distinct type of input. Coherence above 10 Hz was rare, but there may have been some small task-related differences in both unilateral and bilateral motor unit pairs at higher frequencies. Although simulation studies (Lowery et al. 2007; Moritz et al. 2005) and experimental findings (Farmer et al. 1993, 1997; Halliday et al. 1999; Kilner et al. 2002; Semmler et al. 2002, 2004) indicate a close connection between short-term
synchronization and coherence in the beta and gamma frequency ranges, we found the K’ index to be far more sensitive as a measure of short-term synchrony than high frequency coherence.

In addition to characterizing descending drives onto the HMN, our results provide a starting point for more detailed investigations, particularly within a volitional framework (e.g. breath control, speech vs. non-speech movement), and in clinical populations (e.g. tremor, stuttering). Further, motor unit coordination within the right and left bellies of the GG during speech or speech-like movement may reflect the lateralization of cortical activity associated with control of speech-related muscles (e.g. Simonyan et al. 2009; Sowman et al. 2009). Respiration-related control of the HMN may be further probed by experimentally manipulating inspired gas composition or airway resistance. Finally, it would be informative to compare common modulation of GG motor units to other muscles of the tongue, which do not play a central role in airway maintenance, and whose motor units receive bilateral input from the motor cortex.

Overall, we found that in motor unit pairs recorded from the GG, coordination of spike timing and firing rates characterize a unilateral cortical input to the HMN in tongue protrusion, while a bilaterally distributed common drive characterizes synaptic input to the HMN in rest breathing. These results provide a basis for further investigation of synaptic drive onto the HMN through measures of motor unit coordination and suggest that the HMN may be a particularly useful target of such study given the diversity and experimental manipulability of its inputs.
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References


Tables:

Table 1

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Table 1: Common drive index (CDI) and short-term synchrony (K') measures across experimental conditions. Unilateral recordings taken during volitional tongue protrusion had the largest common drive and short-term synchrony measures.
**Figure Legends**

**Figure 1.** Evaluation of common drive, short-term synchrony, and coherence using a pair of motor units recorded during tongue protrusion. Panel A shows a 10 s epoch of discriminated motor unit activity for two simultaneously active GG motor units. To the right of each spike train is an expanded time view showing the superimposition of 50 consecutive action potential waveforms. Smoothed firing rate traces (Panel B) were constructed for the same motor unit pair. Smoothed firing rate traces were filtered (.75 Hz) and used to construct a cross-correlogram, shown in Panel C. The Common Drive Index (CDI) was calculated as the peak correlation coefficient within 100ms of the zero lag. To evaluate short-term synchrony, the spike timings were used to construct a cross-correlation histogram (Panel D, bottom), whose peak-region is defined using the cumulative sum derivative (CUSUM) of the histogram (Panel D, top). The ratio of spikes within the peak-region (vertical lines) relative to that expected by chance (off-peak region), yields the K’ index of short-term synchrony. In the coherence analysis (Panel E), the strength of linear correlation between the two signals at each frequency was calculated. The horizontal line shows the 95% confidence level for significant coherence.

**Figure 2.** Differences in short-term synchrony and common drive across task conditions. Panel A shows how common drive, quantified as the common drive index (CDI), varied across recording conditions. The distribution of CDI values are displayed as a box-and-whisker plots, with each plot showing the median CDI value (midline), inter-quartile range (box and whiskers), and outliers (+ signs). Statistical comparison across groups was accomplished using a Kruskal-Wallis ANOVA with Tukey’s HSD post-hoc tests for pairwise comparisons. Significant differences between groups are marked with an asterisk (*). Panel B shows the results of the short-term synchrony analysis, quantified by the K’ index. The highest CDI and K’ values were measured from unilaterally located units recorded during tongue protrusion.

**Figure 3.** Incidence and strength of coherence. Panels A and B compare unilateral motor unit pairs recorded during tongue protrusion (black, N=25) and rest breathing (gray, N=20). Panels C and D compare bilateral motor unit pairs recorded during tongue protrusion (black, N=23) and rest breathing (gray, N=23). The top plots in panels A and C are coherence histograms depicting the number of motor unit pairs which showed above-chance levels of coherence (p<0.05) at each frequency. The coherence histograms compared in panels A and C were tested for statistical differences at each frequency using Fisher’s exact test. The resulting p-values are plotted below the histograms. Horizontal dashed lines mark the p<0.05 and p<0.01 significance levels. The coherence profiles in Panels B and D compare the average coherence values for motor unit pairs within each group. To compare coherence magnitudes across groups, individual coherences were
normalized using Fisher’s z-transform and weighted in proportion to recording duration. The resulting values were either averaged and re-transformed back to coherence (shown in Panels B and D), or directly compared across groups using a two-sample t-test. All conditions showed high levels of coherence at frequencies under 5 Hz in large proportions of the recordings. Both the incidence and strength of coherence at frequencies under about 10 Hz was strongest for unilateral motor units recorded during tongue protrusion. In unilateral recordings, the most extreme difference between tongue protrusion and rest breathing was at 3 Hz for both types of analyses.
Figure 3
APPENDIX B: SYNCHRONIZATION OF PRESYNAPTIC INPUT TO MOTOR UNITS OF THE TONGUE, INSPIRATORY INTERCOSTAL AND DIAPHRAGM MUSCLES

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ABSTRACT

The respiratory central pattern generator (CPG) distributes rhythmic excitatory input to phrenic, intercostal and hypoglossal premotor neurons. The degree to which this input shapes motor neuron activity can vary across respiratory muscles and motor neuron pools. We evaluated the extent to which respiratory drive synchronizes the activation of motor unit pairs in tongue (genioglossus, hyoglossus) and chest wall (diaphragm, external intercostals) muscles using coherence analysis. This is a frequency domain technique, which characterizes the frequency and relative strength of neural inputs that are common to each of the recorded motor units. We also examined coherence across the two tongue muscles, as our previous work shows that, despite being antagonists, they are strongly coactivated during the inspiratory phase, suggesting that excitatory input from the premotor neurons is distributed broadly throughout the hypoglossal motoneuron pool. All motor unit pairs showed highly correlated activity in the low frequency range (1-8 Hz), reflecting the fundamental respiratory frequency and its harmonics. Coherence of motor unit pairs recorded either within or across the tongue muscles was similar, consistent with broadly distributed premotor input to the hypoglossal motoneuron pool. Interestingly, motor units from diaphragm and external intercostal muscles showed significantly higher coherence across the 10-20 Hz bandwidth than tongue muscle units. We propose that the lower coherence in tongue muscle motor units over this range reflects a larger constellation of presynaptic inputs, which collectively lead to a reduction in the coherence between hypoglossal motoneurons in this frequency band. This, in turn, may reflect the relative simplicity of the respiratory drive to the diaphragm and
intercostal muscles, compared to the greater diversity of functions fulfilled by muscles of the tongue.

**INTRODUCTION**

Inspiratory muscles of the thorax (e.g., diaphragm, external intercostal muscles) and upper airway (e.g., tongue, pharyngeal and laryngeal muscles) are driven by motoneurons that generate bursts of action potentials during the inspiratory phase of the respiratory cycle, with no or little activity during the expiratory phase. These bursts of activity are driven by long time scale (e.g., 1-2 Hz in rodents), synchronous activation of motoneurons by the respiratory central pattern generator, presumed to lie in the preBotzinger complex (Peever and Duffin 2001; Peever et al. 2002; Sebe and Berger 2008; Sebe et al. 2006). In addition, synchronized oscillations in medium (15-50 Hz) and high (50-120 Hz) frequency ranges have been widely reported in pairs of inspiratory muscle motoneurons, and in brainstem interneurons with both inspiratory and expiratory discharge patterns (Funk and Parkis 2002; Huang et al. 1993; Huang et al. 1996; Parkis et al. 2003; Peever et al. 2002; Sebe and Berger 2008; van Brederode and Berger 2008). Most studies of synchronous oscillatory activity in respiratory muscle motoneurons have utilized recordings of left and right muscle nerves from hypoglossal, intercostal or diaphragm motoneuron pools. These recordings provide useful information on the presynaptic modulation of an entire motoneuron pool, but do not provide insight into the presynaptic control of identified muscles, or features of the presynaptic input that is shared by pairs of motoneurons. For example, each hypoglossal nerve contains the axons of motoneurons innervating seven different tongue muscles, each with different
mechanical actions and therefore different force and motor unit recruitment requirements. Similarly, intercostal nerves contain axons that innervate muscles with both inspiratory (external intercostals) and expiratory actions on the thorax (internal intercostals and triangularis sterni). Coherence analysis is a method that examines the correlation between two spike trains, but in the frequency domain rather than the time domain. It provides an index of the strength of in-phase (i.e., synchronous) oscillations at each frequency examined. Because the method used to compute coherence removes the autospectra of the individual spike trains, in phase oscillations that are common to each of the neurons are widely believed to reflect pre-synaptic inputs (Farmer et al. 1993; Farmer et al. 1997; Fellous et al. 2003; Fellous and Sejnowski 2000; Fellous et al. 2004; Kriener et al. 2008; Sejnowski and Paulsen 2006; Tetzlaff et al. 2008) (and see Discussion). Whether the nature of the presynaptic input to “hypoglossal” or “intercostal” motoneurons varies across the specific muscles controlled by these motoneuron pools is unknown.

Accordingly, the goal of this study is to examine the nature of the respiration-related presynaptic input that is shared by motor unit pairs in external intercostal muscles, the diaphragm and two identified tongue muscles (the genioglossus and hyoglossus) with opposing mechanical actions. Comparisons were made within muscles (i.e., recording of two motor units from the same muscle), and also across the genioglossus and hyoglossus muscles (i.e., one motor unit from the genioglossus muscle, one motor unit from the hyoglossus muscle). This approach provided novel information on the composition and
distribution of respiration-related presynaptic inputs driving identified muscle motoneuron pools.

METHODS

Experimental preparation. Studies were done in 61 male Sprague-Dawley rats weighing between 300 and 400 grams, and all procedures were approved by the IACUC of The University of Arizona. Anesthesia was initiated by placing animals in a Plexiglas chamber gassed with 3% halothane in oxygen. After induction, the animal was removed from the chamber but received the same anesthetic mixture via a nose cone. A polyethylene catheter was inserted into a femoral vein for the administration of drugs and fluids. As previously described (Bailey et al. 2001; Fuller et al. 1998; Fuller and Fregosi 2000; Fuller et al. 1999), the isoflurane dose was progressively reduced in exchange for deep urethane anesthesia administered intravenously to a final concentration of 1.3 g/kg, with anesthetic depth monitored by applying deep pressure to the paws. Supplemental doses (0.25 g/kg) of urethane were given as needed to maintain analgesia. Colonic temperature was monitored and maintained between 37 and 38 °C with a thermoprobe and temperature sensor connected to a servo-controlled heating pad. The genioglossus, hyoglossus, external intercostal and diaphragm muscles were surgically exposed but left intact, as described previously (Bailey et al. 2001; Fuller et al. 1998; Fuller and Fregosi 2000; Fuller et al. 1999; Janssen and Fregosi 2000).
Electrophysiology. Motor unit potentials were recorded with high impedance (10 Mohm) tungsten electrodes (Frederick Haer, Bowdoin, ME), and were differentially amplified (Grass, model 7WU16K), filtered between 300 and 10,000 Hz and monitored on a storage oscilloscope and computer screen (John et al. 2005). Amplifier output of the filtered motor unit action potentials were sent to an analog-to-digital converter, which sampled each channel at 20,000 Hz, with all data written to the computer’s hard drive, and subsequently backed up on CD ROM discs.

In all experiments, two microelectrodes were inserted into a single muscle using micromanipulators (Narishige) to study within-muscle events, or into each of two different muscles to examine across-muscle events. Based on several years of experience with these muscles in the rat model, we have learned to limit the penetration of the microelectrodes to distances that are less than the thickness of the muscle (John et al. 2005). Once we identified consistent discharge from two motor units on both electrodes (see Fig. 1), a 10 -15 minute recording period commenced. We then moved one or both electrodes, to record from a presumptively different motor unit pair, and the protocol was repeated. We conducted post mortem analysis at the conclusion of six experiments to confirm electrode placement within the targeted muscle.

Motor unit discrimination. Motor unit potentials (Fig. 1) were discriminated on the basis of waveform shape and amplitude, (Spike II software, Cambridge Electronic Design, UK), as described previously (John et al. 2005). In the urethane-anesthetized rat, the bursts of activity recorded from the tongue, diaphragm and intercostal muscles are purely inspiratory (Bailey and Fregosi 2004; 2006; Bailey et al. 2005; Bailey et al. 2001; Fuller
To compute discharge rate we calculated the inverse of the mean interspike interval of all action potentials generated during each burst, obtained the average frequency of each burst, and then averaged across 20 such bursts for each motor unit. To obtain an estimate of discharge rate variability, we computed the coefficient of variation of the interspike intervals for each motor unit. This was done by dividing the standard deviation of the interspike interval by the average interspike interval for that unit, and expressing the data as a percentage. Cycles containing non-respiratory behaviors such as swallows or sighs, which are easy to identify because they cause obvious changes in discharge as well as prolongation of the expiratory period (Janssen et al. 2000) were excluded from analysis.

**Coherence analysis.** Coherence analysis is a frequency domain technique that is commonly applied to dual motor unit recordings to reveal the frequency of oscillations that are common to each of the motor units (Farmer et al. 1993; Farmer et al. 1997; Myers et al. 2004). Computation of the coherence function produces a dimensionless number between 0 and 1, which reflects the strength of the correlation between the activities of the two actively discharging motor units at each frequency. Spike times of each unit were transformed into a continuous signal (sampling rate 1,000 Hz) with each spike represented as a 1 ms pulse. The coherence between two spike trains was calculated with Matlab software using un-weighted, non-overlapping data segments 2048 ms in length, resulting in a frequency resolution of 0.49 Hz. These data were used to compute the magnitude-squared coherence at each 0.49 Hz interval over a frequency
range of 0 - 500 Hz. However, because we found no evidence of coherent discharge at frequencies above 50 Hz, we focus on the 0 - 50 Hz frequency range.

Statistical evaluation of coherence was done in two ways. First, we analyzed the proportion of motor unit pairs showing significant coherence at each frequency between 0 and 50 Hz. To do this, we first obtained the 95% confidence level for each motor unit pair according to the equation \( 0.05^{1/(N-1)} \) where \( N \) is the number of disjoint time segments used in the coherence estimation (Amjad et al. 1989; Rosenberg et al. 1989).

We then derived the proportion of motor unit pairs showing significant coherence at each frequency, and subsequently compared this result both within and across muscles using the chi–squared test, followed by post-hoc comparisons with Fisher’s exact test.

Second, the magnitude-squared coherence for all motor unit pairs was derived by converting raw coherence values into Z-scores using Fisher’s transform \( z = \text{atanh}(\sqrt{C}) \), where \( C \) represents the magnitude-squared coherence; for simplicity, in the remainder of the manuscript magnitude-squared coherence is referred to as “coherence magnitude”. The Z scores for each motor unit pair were then averaged across 2 Hz bins, and a one-way ANOVA was used to compare the values in each frequency bin. Two-tailed, unequal variance t-tests were used for pair-wise post-hoc comparisons. Finally, for each motor unit pair, the maximum un-binned Z-value was calculated for the 10-20 Hz frequency band, in order to test for correlation between coherence and the geometric mean firing rate of each motor unit pair.

RESULTS
Number of motor unit pairs and average motor unit discharge rates. We recorded the activity of 167 motor unit pairs from 61 animals. Within muscle comparisons included recordings from 33 genioglossus motor unit pairs, 54 hyoglossus pairs, 14 external intercostal muscle pairs and 13 diaphragm motor unit pairs. We also studied 22 hyoglossus-genioglossus pairs. We used an average of 7344 ± 5690 (mean ± SD) spikes from each motor unit to construct the coherence spectra. The average breathing frequency across all animals ranged from approximately 80 to 100 cycles per minute (93.81 ± 20.31, mean ± SD), or 1.33 to 1.66 Hz, consistent with findings reported previously in spontaneously breathing urethane anesthetized rats (Bailey and Fregosi 2004; Bailey et al. 2006; Bailey et al. 2005; Bailey et al. 2001; Fregosi and Fuller 1997; Fuller et al. 1998; Fuller and Fregosi 2000; Fuller et al. 1999; Janssen et al. 2000).

For all muscles, single motor unit activities exhibited consistent, inspiratory-related activity, and all recordings were characterized by high signal to noise ratios (see Fig. 1). Average inspiratory-related discharge rates (mean ± SD) were 42.3 ± 7.9 Hz (N=78), 52.3 ± 11.6 Hz (N=128), 36.8 ± 7.8 Hz (N= 30) and 31.5 ± 6.1 Hz (N=32) for genioglossus, hyoglossus, intercostal and diaphragm motor units, respectively. One way ANOVA revealed significant differences in inspiratory-related discharge rate (F = 53.65, P<0.001) with hyoglossus > genioglossus > intercostal and diaphragm (all P<0.001 by Bonferroni post hoc tests).

Discharge rate variability. We determined within muscle discharge rate variability by computing the coefficient of variation of the interspike intervals for all motor units (Fig. 2). ANOVA revealed significant differences (F=24.21, P=0.0018) between the
diaphragm and genioglossus (P<0.001), diaphragm and hyoglossus (P<0.001) and diaphragm and external intercostal muscles (P<0.001), however there were no differences between intercostal and either genioglossus or hyoglossus muscles, or between genioglossus and hyoglossus muscles.

**Coherence of motor units within a muscle.** Simultaneous recordings of two motor units within a muscle allowed us to determine the proportion of that muscle’s motor unit pairs that show coherent discharge at each frequency from 0-50 Hz (Fig. 3A). For all four muscles, the proportion of coherent motor unit pairs was very high at low frequencies and fell monotonically from approximately 8- to 50 Hz. The curves for intercostal and diaphragm muscle motor units were right shifted compared to the tongue muscle curves, such that the frequency at which 50% of the motor unit pairs showed significant coherence was 12 and 15 Hz for genioglossus and hyoglossus, vs. 23 and 30 Hz for the intercostal and diaphragm muscle motor units.

Average coherence magnitudes for motor unit pairs recorded within a muscle are shown over the 0-50 Hz frequency range in Fig. 3B. Note that all muscles showed very high coherence values over the 1-8 Hz bandwidth, reflecting the fundamental respiratory burst frequency and the first three-four harmonics of that frequency, as shown by analysis of the power spectra of individual motor units (see representative examples in Fig. 4).

The average coherence magnitude of motor unit pairs from each of the four muscles was compared statistically by converting raw coherence values to Z scores followed by one-way ANOVA (see Methods), and the results are provided in the bottom panel of Fig. 3B. ANOVA revealed systematic and highly significant differences
throughout the 10- to 20 Hz frequency band, while post hoc analyses confirm that the 10-20 Hz differences were dominated by contrasts between tongue and chest wall muscles.

**Coherence of motor units across the genioglossus and hyoglossus muscles.** The proportion of genioglossus-hyoglossus motor unit pairs showing significant coherence at each frequency is shown in Fig. 5A. As for the within muscle comparisons shown in Fig. 3A, the proportion of coherent motor unit pairs across the genioglossus and hyoglossus muscles was very high at low frequencies, but in this case fell more sharply. The frequency at which 50% of the motor unit pairs showed significant coherence was in the range of 9-11 Hz. Coherence magnitude between hyoglossus and genioglossus muscle motor units falls off very sharply at frequencies above 8 Hz, suggesting that almost all of the coherent synchronization of motor units in the genioglossus and hyoglossus muscles arises in the respiratory central pattern generator (Fig. 5B).

**Coherence: comparison of tongue muscles with muscles of the chest wall.** We combined all motor unit pairs recorded within each of the tongue muscles and all motor unit pairs recorded within each of the chest wall muscles to assess differences in motor unit coherence between spinal and cranial motoneurons (Fig. 6). Motor units of chest wall muscles are more likely to show coherent oscillations than are tongue muscle motor units over the 10-40 Hz bandwidth (Fig. 6A). The frequency where 50% of the pairs showed significant coherence was about 13 Hz for tongue muscle motor units, and 26 Hz for motor units from the chest wall muscles. Significant differences in coherence magnitude between tongue and chest wall motor units were detected in the 3-5 Hz and 10 to 20 Hz bandwidths, with chest wall motor unit pairs exhibiting significantly higher
coherence values than tongue muscle motor unit pairs (Fig. 6B). Since previous studies in limb muscles show that the computation of coherence can be influenced by discharge rate (Christou et al. 2007; Lowery and Erim 2005), we examined the relationship between coherence magnitude and firing rate for all motor unit pairs by transforming coherence into Fisher’s Z scores, computing the geometric mean discharge rate for each pair of motor units, and performing a Pearson correlation analysis. As shown in Fig. 7, the relationship between coherence and discharge rate is flat ($r^2 = 0.00010$).

Because we did not measure blood gases in our experiments it is possible that changing anesthetic levels and thus blood gases, both within and across experiments, may have biased our results. Accordingly, for each pair of motor units studied, we measured the animal’s respiratory frequency as an index of anesthetic depth, and computed the correlation between the magnitude-squared coherence of each motor unit pair, and the respiratory frequency. The results of that analysis show no relationship between breathing rate and coherence in the 10-20 Hz range ($r^2 = 0.0008$, $P = 0.76$). In addition, to insure that there were no systematic differences in anesthetic depth during recordings of chest wall and tongue muscle motor unit pairs, we compared the average breathing frequency associated with all recordings of diaphragm, intercostal, hyoglossus and genioglossus motor unit pairs. The results of that analysis also revealed no significant differences ($F = 0.169$, $P = 0.174$).

**DISCUSSION.**
**Summary.** Phasically driven motor units in muscles of the tongue and chest wall show strong correlated activity at frequencies between approximately 1.5 and 8 Hz. Coherence at these low frequencies represents synchronized presynaptic oscillations at the fundamental frequency of the respiratory central pattern generator, and harmonics of this fundamental frequency. These observations were expected, as in our preparation the motor units are driven spontaneously by an exceptionally strong, stereotyped input function that is distributed concurrently to spinal (intercostal, diaphragm) and cranial (tongue muscles) motoneurons. While there were small differences in the coherence profiles of tongue vs. chest wall motor units in the 3-5 Hz range, the largest and most consistent differences were between about 10 and 20 Hz, with chest wall muscle motor units showing higher levels of coherence throughout this frequency band. We propose that the lower coherence in tongue muscle motor units over this range reflects a larger constellation of presynaptic inputs, which collectively lead to a reduction in the coherence between hypoglossal motoneurons in this frequency band. This, in turn, may reflect the relative simplicity of the respiratory drive to the diaphragm and intercostal muscles, compared to the greater diversity of functions fulfilled by muscles of the tongue.

**Motor unit discharge rates and variability.** Tongue muscle motor units had significantly higher discharge rates than the two chest wall muscles, with the hyoglossus muscle having the highest of all, and the diaphragm the lowest. This is consistent with recent data in human subjects, showing rates of 10-18 Hz in diaphragm, 8-11 Hz in external intercostal muscles and 14-30 Hz in the genioglossus muscle (Saboisky et al. 2007a; Saboisky et al. 2007b). In adult rats, the input resistance of phrenic and
hypoglossal motoneurons is about 2 and 12 Mohms, respectively (Hayashi and Fukuda 1995; Viana et al. 1995). Similarly, rheobase current ranges from 5-14 nA in phrenic motoneurons (Jodkowski et al. 1987), and 1-2 nA in hypoglossal motoneurons (Takata et al. 1980). Thus, equivalent levels of synaptic input should result in higher discharge rates in hypoglossal motoneurons, as they are intrinsically more excitable.

Interestingly, the coefficient of variation of motor unit discharge rates is also significantly lower in the diaphragm compared to the other muscles. Given that the variability of interspike intervals increases as a function of the amplitude and frequency content of synaptic noise and the duration of the after-hyperpolarization current (Powers et al. 2002), this observation is consistent with increased synaptic noise and/or different intrinsic motoneuron properties in tongue compared to diaphragm muscles. Taken together, these observations suggest that motor units with lower intrinsic excitability have slower and more uniform firing profiles. Interestingly, using our average firing rate data together with published data on the proportion of type I muscle fibers for each of the muscles yields an inverse, monotonic relationship (Fig. 8). It is noteworthy that the tongue muscles have either no type I fibers (hyoglossus) or just a few (genioglossus). Inasmuch as histochemical fiber type correlates with muscle shortening velocity, axon diameter and input resistance (Sawczuk et al. 1995). This relationship is expected, but is consistent with the significant differences in firing rates in the tongue and chest wall muscles that we observed here.

Coherence of motor units within a muscle. Previous studies have examined the correlated discharge of pairs of motor units during volitional contractions in human
subjects, and used coherence analysis to estimate the extent to which in-phase oscillations synchronize motoneuron discharge (Baker et al. 1999; Farmer et al. 1997; Laine and Bailey; Lowery et al. 2007). Similarly, in vitro studies show that in-phase oscillations in the 20-50 Hz range are involved in spike timing precision in phrenic motoneurons (Parkis et al. 2003), and that the reliability of action potential discharge in hypoglossal motoneurons is highest when the input frequency of an injected sinusoid is in the 3-25 Hz range (van Brederode and Berger 2008). Our data are consistent with these observations inasmuch as the frequency at which 50% of the motor unit pairs showed significant coherence is about 13 and 26 Hz for tongue and chest wall muscles, respectively (Fig. 6A). However, we have also demonstrated that the incidence and magnitude of inspiratory-phase coherence in the 10-20 Hz bandwidth is consistently and significantly greater in muscles of the chest wall compared to muscles of the tongue (Fig. 6A&B). Although the reason for this difference is unknown, it is clear that statistically significant coherence in this frequency band would require a reasonably strong in-phase oscillation that is common to each of the motor units being analyzed. However, if one motoneuron pool receives more sources of input which contain activity in the 10-20 Hz range, and if those inputs arrive out of phase with each other, they will summate destructively, leading to relatively weaker coherence. Based on this idea, we suggest that in addition to the inputs from the respiratory central pattern generator that appears to be shared equally by both motoneuron pools, the hypoglossal motoneurons receive a wider constellation of presynaptic inputs than phrenic/intercostal motoneurons, possibly due to the wider range of functions performed by tongue muscles compared to diaphragm or intercostal muscles.
Some evidence for this conclusion includes robust, inspiratory-phase GABAergic and glycinergic inputs to hypoglossal motoneurons from the reticular formation (Fenik et al. 2004; Fenik et al. 2005; O'Brien et al. 2004; Remmers et al. 1980; Withington-Wray et al. 1988; Woch and Kubin 1995) and also the nucleus of Roller (Marchetti et al. 2002; O'Brien et al. 2004), which is a relay site for sensory afferents. Importantly, our previous work in the same preparation showed that pulmonary stretch receptors evoke much stronger inhibition of tongue compared to intercostal muscles (Bailey et al. 2001; Fregosi and Fuller 1997; Janssen et al. 2000), consistent with stronger inhibitory synaptic inputs to hypoglossal compared to intercostal motoneurons.

Coherence between genioglossus and hyoglossus muscle motor units. In the last decade, our laboratory has documented respiratory-related co-activation of protrudor and retractor tongue muscles in the rat (van Brederode and Berger 2008), observations that were subsequently confirmed in human subjects (Bailey and Fregosi 2004; Bailey et al. 2005; Bailey et al. 2001; Fuller et al. 1998; Janssen and Fregosi 2000; Janssen et al. 2000). The present results show very low coherence at frequencies above those that are due to the fundamental respiratory frequency and the first few harmonics of this fundamental frequency. These results suggest that presynaptic input from the central pattern generator is distributed broadly to the hypoglossal premotor neuron pool, leading to synchronized excitation of hypoglossal motoneurons that drive both protrudor and retractor muscles of the tongue. Previous anatomic studies have shown that motoneurons driving the genioglossus and hyoglossus muscles in the rat are somatotopically organized within the hypoglossal motor nucleus (Mateika et al. 1999), but we do not know if the
hypoglossal premotor neurons that convey excitatory synaptic input from the respiratory central pattern generator have unique projections to genioglossus and hyoglossus motoneurons, or if they branch extensively to innervate motoneurons in two or more muscle motoneuron pools. Peever et al (2001) used cross correlation analysis of inspiratory bursts in medial and lateral hypoglossal nerve branches (which innervate the genioglossus and hyoglossus muscles, respectively), and found strongly correlated activity. In contrast, they failed to find significant correlations between hypoglossal and phrenic nerve activities. They interpreted the data as evidence for a common drive from the hypoglossal premotor neuron pool, which agrees with our findings of similar coherence profiles among GG and HG motor units. These observations and the present ones suggest that excitatory synaptic input from the respiratory central pattern generator is distributed broadly to the hypoglossal premotor neurons, leading to respiration-related coactivation of genioglossus and hyoglossus muscles.

**Conclusions.** Phasically driven motor units in muscles of the tongue and chest wall show strong correlated activity at frequencies between approximately 1.5 and 8 Hz, reflecting presynaptic oscillations from the respiratory central pattern generator and harmonics of this fundamental frequency. Chest wall muscle motor units consistently showed higher levels of coherence than tongue muscle motor units over the 10-20 Hz frequency band. We propose that the lower coherence in tongue muscle motor units over this range reflects a larger constellation of presynaptic inputs, which collectively lead to a reduction in the coherence between hypoglossal motoneurons in this frequency band. This, in turn, may reflect the relative simplicity of the respiratory drive to the diaphragm.
and intercostal muscles, compared to the greater diversity of functions fulfilled by muscles of the tongue.
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Figure Legends.

1. Representative dual motor unit recordings from within four muscles: the genioglossus (GG-GG), hyoglossus (HG-HG), inspiratory intercostal (IC-IC) and diaphragm (Dia-Dia). The phasic discharge is confined largely to the inspiratory phase of the respiratory cycle.

2. The coefficient of variation (CV) of the interspike interval (ISI) for all recorded motor units from the genioglossus (GG), hyoglossus (HG), inspiratory intercostal (IC) and diaphragm muscles. The horizontal bars indicate the mean value. The CV of ISI in diaphragm motor units was significantly lower than in all other muscles. ***, P<0.001 vs. diaphragm.

3. Proportion of coherent motor unit pairs and average coherence magnitude for all within muscle comparisons. Panel A shows the proportion of within muscle motor unit pairs showing significant coherence at each frequency, for all four muscles. The proportion of coherent motor unit pairs in each of the four muscles, and at each frequency was compared statistically (see Methods), and the results of this analysis is depicted as P values in the lower section of Panel A. Panel B shows the average coherence magnitude between motor unit pairs within each of the muscles, with the P values derived from one-way ANOVA located beneath the coherence data. There were significant differences in coherence magnitude across the 9-20 Hz bandwidth.

4. Power spectral density of representative single motor unit discharges from each of the muscles. Note the large power values at the fundamental respiratory frequency and the first 2-3 harmonics of this fundamental frequency. The power in the 30-60 Hz range reflects the approximate mean firing rate of the motor units.

5. Proportion and average coherence magnitude for motor units recorded simultaneously form the GG and HG. Panel A shows the proportion of GG-HG motor unit pairs showing significant coherence at each frequency. Panel B shows the average coherence magnitude coherence between GG-HG motor unit pairs. Both the proportion of coherent GG-HG pairs and the coherence magnitude for these across muscle comparisons are very similar to the within muscle comparisons (GG-GG and HG-HG) shown in Fig. 3A.

6. The proportion (Panel A) and magnitude (Panel B) of coherence in motor unit pairs from tongue and chest wall muscles. For this analysis we combined all motor unit pairs from both tongue muscles, and all pairs from the two chest wall muscles, to compare the proportion and magnitude of coherence in cranial and spinal motoneurons. Note that coherent oscillations in chest wall muscle motor units are significantly higher than tongue muscle units over the 8-20 Hz bandwidth.
7. Coherence magnitude (transformed into Fisher’s Z scores) as a function of discharge rate for all motor unit pairs studied. The mean discharge rate was computed as the geometric mean of the average discharge rate of each motor unit in a pair. The regression line and $r^2$ values are shown.

8. Relationship between the average discharge rate that we recorded in rodent hyoglossus, genioglossus external intercostal and diaphragm muscles, and the percentage of Type I muscle fibers in each muscle, as reported by others (Cunningham et al. 1991; LaFramboise et al. 1992; Prakash et al. 2000; Smith et al. 2005; Sutlive et al. 2000).
Figure 2
Figure 3

A

B

P value

Proportion

Coherence

frequency (Hz)

GG
HG
IC
DIA
Figure 4
Figure 5
Figure 7

$r^2 = 0.00010$

Fisher's $Z$

frequency (Hz)
Figure 8
APPENDIX C: CORTICAL ENTRAINMENT OF HUMAN HYPOGLOSSAL MOTOR UNIT ACTIVITIES

Abbreviated title: cortical entrainment of motor units

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Abstract

Output from the primary motor cortex contains oscillations which can have frequency-specific effects on the firing of motoneurons (MNs). While much is known about the effects of oscillatory cortical drive on the output of spinal MN pools, considerably less is known about the effects on cranial motor nuclei which govern speech/oromotor control. Here we investigated cortical input to one such motor pool, the hypoglossal motor nucleus (HMN), which controls muscles of the tongue. We recorded intramuscular genioglossus EMG and scalp EEG from healthy adult subjects performing a tongue protrusion task. Cortical entrainment of HMN population activity was assessed by measuring coherence between EEG and multi-unit EMG activity. In addition, cortical entrainment of individual MN firing activity was by measuring phase-locking between single motor unit (SMU) action potentials and EEG oscillations. We found that cortical entrainment of multi-unit activity was detectable within the 15-40 Hz frequency range, but was inconsistent across recordings. By comparison, cortical entrainment of SMU spike-timing was reliable within the same frequency range. Further, this effect was found to be intermittent over time. Our study represents an important step in understanding corticomuscular synchronization in the context of human oromotor control, and is the first study to document SMU entrainment by cortical oscillations in vivo.
Introduction

The cortical signals that produce voluntary muscle contraction have traditionally been investigated *in vivo* by characterizing the degree to which cortical drive coordinates groups of motoneurons (MNs). Of particular interest is the ability of oscillatory components of cortical drive to entrain the output of motor pools. This phenomenon is reflected by frequency-specific (~15-40 Hz) synchronization between cortical activity and whole muscle EMG (Conway et al., 1995; Salenius et al., 1997; Brown et al., 1998; Halliday et al., 1998; Gross et al., 2000); for reviews see (Mima and Hallett 1999; Schnitzler et al. 2000; Grosse et al. 2002).

The functional significance of such entrainment is not entirely clear, and may represent only one facet of corticomuscular communication. Simulation studies and experiments carried out *in vitro* suggest that individual MNs intrinsically respond more strongly and with greater temporal precision to oscillatory input when it is delivered at specific frequencies (also ~15-40 Hz)(Hunter et al. 1998; Funk and Parkis 2002; Parkis et al. 2003; van Brederode and Berger 2008). Physiologically, it might improve input-output efficiency within the motor system if cortical drive contained oscillatory components at frequencies that optimally activate MNs.

In the present investigation, we have studied the effects of oscillatory cortical drive on the hypoglossal motor nucleus (HMN), which innervates the muscles of the tongue. The human HMN not only receives direct projections from the primary motor cortex (Snell 1980), but also diffuse inputs arising from respiratory-related central pattern generation and afferent feedback (reviewed in Sawczuk and Mosier 2001) from a variety
of sources. The array of non-cortical sources of input may interfere with 15-40 Hz synchronization of MNs, or reduce the ability of standard methods to detect it. Although there is evidence to suggest that during voluntary activation, hypoglossal MNs are not strongly synchronized by shared oscillatory input >~10 Hz (Laine and Bailey 2011), synchronization between cortical EEG and MN population activity has not directly been investigated. An alternative and potentially more sensitive means of examining cortical entrainment of MN activities would be to evaluate the timing of single motor unit (SMU) action potentials relative to cortical oscillations. Others have shown in vitro that hypoglossal MN spike-timing becomes tightly entrained (phase-locked) to oscillatory stimulation at frequencies falling within the 15-40 Hz range (van Brederode and Berger 2008). To date, this effect has not been confirmed in vivo or within the context of cortical drive to MNs. Such frequency-specific effects on spike-timing may have implications related to the efficiency of neural responses to synaptic input (Funk and Parkis 2002; Parkis et al. 2003) and could increase our understanding of how cortical oscillations entrain (or fail to entrain) populations of MNs.

Accordingly, we characterized cortical effects on hypoglossal MN activities at the level of individual MNs (cortical entrainment of SMU spike-timing), as well as effects on the population of MNs as a whole (cortical entrainment of multi-unit EMG activity). We found that cortical oscillations in the 15-40 Hz range entrain hypoglossal MN activities, with the strongest effects occurring at about 20 Hz. Importantly, our findings show that entrainment of SMU spike-timing by cortical oscillations is detectable in vivo,
and in this case, provides a more robust measure of cortical drive than entrainment of multi-unit EMG activity.

**Methods**

All procedures were approved by the Human Subjects Committee at the University of Arizona. Recordings were made as subjects sat upright in a dental chair and maintained tongue protrusion for approximately 2 minutes (118.3 ± 32.4 s for single motor unit recordings and 121.88 ± 18.0 s for multi-unit recordings).

EMG recordings:

For each subject, the depth to the inferior border of the target muscle m. genioglossus (GG) initially was determined using ultrasonography (Pro Sound 3500, Aloka, Tokyo, Japan) (Eastwood et al. 2003). Intramuscular EMG recordings subsequently were obtained using tungsten needle electrodes (100 kΩ, Frederick Haer, Bowdoinham, ME) inserted percutaneously into the right belly of the GG and referenced to the right mastoid process. Subjects were instructed to protrude their tongue from rest position to, or just beyond, the front teeth. In this regard, the degree of muscle activation did not vary systematically as a function of recording type. The type of recording obtained (SMU or multi-unit) depended upon whether the EMG activity adjacent to the electrode tip was dominated by the active fibers of a single motor unit, or instead reflected the combination of dispersed multi-unit activity. All EMG signals were preamplified (10x), amplified (100x), and band-pass filtered (200-2,000 Hz) using CED 1902 amplifiers and headstages (Cambridge Electronic Design, Cambridge UK). The
filter settings we adopted were optimized for analysis of spiking activity embedded within the EMG signals. In the case of multi-unit signals, it has recently been shown experimentally and in simulations that the summed activity of a small number of SMUs can effectively be used in identifying cortical entrainment of motor unit activity across a whole muscle (Negro and Farina, 2011a, 2011b). Also, it has been suggested that the highest frequency components of EMG activity may be the most physiologically relevant for many applications, while lower frequencies can easily be contaminated by artifacts attributable to the filtering effects of skin and soft tissue, movement, and/or the activity in adjacent musculature (Potvin and Brown, 2004; Staudenmann et al., 2007; Riley et al., 2008; Brown et al., 2010). All EMG data were acquired (sampling rate = 25 kHz) and stored using the CED 1401 interface and associated Spike2 software.

Multi-unit EMG recordings:

Multi-unit EMG recordings were obtained from 6 adult subjects (4 male, 2 female). Subjects were asked to protrude the tongue and maintain a steady level of activation using visual feedback of the RMS EMG activity within a 200 ms moving window. Prior to off-line analysis, the raw whole muscle EMG signals were rectified (RMS within a 10 ms moving window) and down-sampled to 1000Hz. Figure 1A displays an example recording in which EEG and whole muscle EMG signals were recorded simultaneously during tongue protrusion. The EEG (top trace) and RMS rectified EMG (bottom trace) were used to calculate EEG-EMG coherence (described below).

Single motor unit (SMU) recordings:
Single motor unit recordings were obtained from 13 adult subjects (6 male, 7 female). Subjects were required to sustain tongue protrusion until a single unit could be isolated, at which point they were asked to maintain the activity using visual feedback from the raw EMG voltage trace containing SMU action potentials. SMU action potentials were discriminated offline using a template matching algorithm provided by the Spike2 software and the results were checked and manually corrected if necessary. Figure 1B displays an example recording in which SMU action potentials present within the EMG (middle trace) were discriminated (lower trace and inset), and tested for phase-locking with EEG activity (described below).

EEG recordings:

Electroencephalographic (EEG) activity was recorded over oral sensorimotor cortical areas using a reference free, 5-point electrode montage centered at C5 of the international 10-20 system. The outer 4 points of the array were positioned 2.5 cm from the central point and at 90 degree increments from each other. This arrangement allowed a single EEG trace to be obtained using the Hjorth transform (surface Laplacian), which amplifies activity within the boundaries of the electrode array while minimizing the influence of volume-conducted noise (Hjorth 1975). EEG activity was preamplified (10x), amplified (100x), and band-pass filtered (1.5-150 Hz) using a CED 1902 headstage and amplifiers. The data was sampled/stored at 1000 Hz using a CED 1401 interface and Spike2 software.

Analysis:
All signal processing and data analysis was carried out offline using Matlab 7.0 (The MathWorks, Natick, MA) and Spike 2 software.

EEG-EMG coherence:

To quantify correlated oscillatory activity between EEG and RMS rectified EMG activities, we calculated coherence. For a given frequency, coherence expresses the correlation between two signals on a scale of 0 to 1, with 1 indicative of perfect linear correlation and 0 indicative of no correlation. Coherence was calculated using the `mscohere` function within Matlab, specifying that data be segmented into non-overlapping, un-weighted segments (2048 ms duration). This yielded coherence values for frequencies up to 500 Hz, with 0.49 Hz resolution. For each EEG-EMG coherence measurement, a 95% confidence level was determined according to the equation 1 - 0.05^[1/(N-1)], where N is the number of disjoint data segments used in the analysis (Carter 1987; Rosenberg et al. 1989). An example coherence profile for a single recording is displayed in Figure 1C. In this example, the strongest coherence between whole muscle EMG and EEG occurred at ~20 Hz.

We conducted group coherence analysis in two ways. The first was to quantify, per frequency, the percent of recordings that showed a statistically significant level of coherence. A binomial test was used to determine the percent that would exceed what could be expected by chance. We also sought to characterize any trends in coherence magnitudes across frequencies. When considering coherence values derived from multiple recordings, Fisher’s Z-transform (Z = atanh(√C), where C = coherence) is typically applied prior to statistical testing (Rosenberg et al. 1989). Accordingly, we
converted coherence values to Fisher’s $Z$ values when displaying population data. Although procedures exist for averaging individual coherence profiles (Kilner et al. 1999), we chose to produce a group coherence profile by calculating “pooled coherence” (Amjad et al. 1997). This procedure is particularly straightforward to interpret, as it equates to concatenating individual recordings and calculating coherence as for a single trial. Prior to pooling the data, we normalized all EMG and EEG recordings to unit variance to reduce potential overestimates of pooled coherence (Baker 2000). For the present study, we restricted analysis to an appropriate frequency range for investigating beta/gamma band effects (less than 100 Hz), and displayed all coherence measures in 1 Hz frequency bins, using the maximum value found within each bin.

SMU-EEG phase-locking:

To investigate timing relationships between EEG and SMU firing, we used wavelet analysis to decompose the EEG signal such that the instantaneous phase of component frequencies could be tracked over time. To do this, we convolved complex Morlet wavelets with the EEG signal to create a time-frequency representation. Each wavelet was created as a Gaussian-windowed complex sinusoid $e^{i2\pi tf} * e^{-t^2/(2*\sigma^2)}$, where $t=$time, $f=$frequency (from 1 to 100 Hz), and where the “width” of each wavelet was constrained to 3 cycles by setting $\sigma = 3/(2\pi f)$. The result of the wavelet convolution is a complex time series for each frequency ($W(t,f)$). The times at which SMU action potentials occurred (rounded to the nearest ms) could then be associated with instantaneous phase values for each frequency component of the EEG. If action potentials occur randomly with respect to the peaks and valleys of a given oscillation, the
distribution of phase values should be uniform. Conversely, if spikes tend to occur at a preferred phase of the oscillation, the distribution of phase values will exhibit a peak, a phenomenon referred to as “phase-locking”. To test the randomness of spiking activity with respect to EEG oscillatory activity, we calculated Rayleigh’s Z (RZ) statistic using the following equation

$$RZ(f) = N \sum_{k=1}^{N} \left| \frac{W_{(k,f)}}{W_{(k,f)}} \right|^2$$

where N= the number of action potentials used in the analysis and k= the time at which each action potential occurred. This measure is closely related to the commonly used Phase Locking Factor (PLF) described by Tallon-Baudry et al. 1996, since RZ= N * PLF^2 (Fisher 1993). Rayleigh Z values were used to enable comparison across datasets having different numbers of action potentials, and direct conversion to p-values using the equation p=e^(-RZ) (Fisher 1993). Accordingly, RZ values ≥ 3 exceed the 95% confidence level. Figure 1D displays an example of SMU-EEG phase locking. In this example, strong phase-locking was present from about 20-25 Hz.

We conducted a group analysis in two ways. First, we determined the percent of motor units showing significant phase-locking to EEG at each frequency and used a binomial test to determine what percent constituted a statistically significant proportion of the recordings. Second, we calculated a group-average Rayleigh Z value for each frequency. A 95% confidence level for this averaged data was derived using a surrogate data set. To do this, the action potentials in each recording were associated with randomized phase-angles and the average Rayleigh Z value was calculated across the
phase-randomized recordings. This procedure was repeated 1000 times, after which a t-test was used to determine the 95% confidence level. Since the firing rates of many motor units were close to the expected frequency range of phase-locking, the distribution of observed phase-values at these frequencies may not be perfectly uniform even under the assumption of randomly-timed spikes. We therefore conducted a more rigorous test by shuffling the spikes within each SMU recording (preserving inter-spike-intervals), and then using a paired t-test to compare the degree of phase-locking observed experimentally to that of the shuffled data set. The merit in spike shuffling has been shown previously for applications that involve statistical analysis of spike train data in the frequency domain (Rivlin-Etzion et al., 2006). This procedure was repeated 30 times, after which a combined p-value was calculated for each frequency using Stouffer’s Z-score method.

We then evaluated the temporal progression of SMU-EEG phase-locking by calculating Rayleigh Z values within a 5-second window translated across each recording with a step-size of 2.5 seconds. We then calculated the percent of time windows in which motor units showed significant levels of phase-locking to EEG at each frequency. This technique also allowed us to determine if phase-locking strength was correlated with temporal fluctuations in EEG power. The average EEG power for each frequency was determined in each time window, and these values were tested for correlation with the associated Rayleigh Z values using Spearman’s rank correlation. The number of statistically significant (p<0.05) positive or negative correlation values obtained across the population of motor units could then be tested against the 5% error level using a binomial test.
Results

A total of 34 multi-unit EMG signals were recorded from 6 subjects, and 63 SMUs were recorded from 13 subjects. Each recording (either SMU or multi-unit) was obtained during a different tongue protrusion trial. The number of SMU or multi-unit recordings obtained per subject ranged between 2 and 10. On average we obtained 4.8 (± 2.9) SMU recordings per subject and 5.6 (± 3.1) multi-unit recordings per subject. Multi-unit data was obtained only from subjects who also provided SMU data.

EMG to EEG coherence:

The results of the EMG to EEG coherence analysis are displayed in Figure 2A. The color of each pixel represents the Fisher-transformed coherence for a given frequency (columns) and recording (rows). Coherence magnitudes were highly variable across frequencies and across recordings, with no clear tendency for particular frequency bands to show stronger coherence. The incidence of significant coherence across the population of recordings is shown in Figure 2B. The highest incidence of significant coherence occurred at 20 Hz. The measure of pooled coherence yielded more clearly distinguishable peaks (see Figure 2C), and showed a significant degree of coherence within the beta/gamma range, with peaks at about 20 and 35 Hz.

SMU to EEG phase-locking:

Figure 2D displays the phase locking profiles for each of the 63 SMU recordings. As shown by the phase-locking profiles in Figure 2D, there was a clear trend towards higher Rayleigh Z values across the beta/gamma range in the majority of motor units. In fact, about 36% of motor units showed statistically significant phase-locking within the
15-35 Hz range. The histogram in Figure 2E quantifies, per frequency, the percent of motor units which showed significant phase-locking to EEG. The peaks centered near 20 and 35 Hz clearly exceed the 95% confidence level. In addition, we calculated a population-average Rayleigh Z value, shown in Figure 2F. The horizontal line marks the 95% confidence level for statistical significance. We also compared the experimentally observed phase-locking data to a dataset created by shuffling each SMU spike train prior to phase-locking analysis. The results of 30 such tests are shown in Figure 3 (top panel). Each time that the SMU spike trains were shuffled (rows), the likelihood that the same overall degree of phase-locking could have occurred by chance (color) was quantified for each frequency (columns). The 30 shuffle tests were then combined in order to obtain a single p-value for each frequency, the results of which are shown in Figure 3 (bottom trace). As shown, the degree of experimentally observed phase-locking is greater than could be obtained by chance throughout the 15-25 Hz frequency range.

In general, the analysis of SMU-EEG phase-locking shown in Figures 2 and 3 indicate a robust and consistent effect of cortical oscillatory activity on the timing of SMU action potentials. This is in contrast to the EEG-EMG coherence data shown in the left column of Figure 2, where individual recordings do not show particularly consistent coherence profiles, and data must be pooled in order to discern any effect of oscillatory cortical drive on the MN population. Even so, beta/gamma band synchronization (peaking at about 20 Hz) between EEG and either whole muscle EMG or SMU spike-timing is clearly a feature of hypoglossal motor control.

Temporal characterization of SMU-EEG phase-locking:
Evaluating SMU-EEG phase-locking over short time periods enabled us to track the temporal progression of the phenomenon on a per-unit and per-frequency basis. An example time-frequency breakdown of SMU-EEG phase-locking is shown in Figure 4A. In this plot, the color of each pixel represents the Rayleigh Z value calculated within a 5 s moving window, with all non-significant Z values set to 0. The beta/gamma band shows the largest and most variable Rayleigh Z values over time. We collapsed this information across time for each recording, and quantified the proportion of time in which significant phase-locking occurred. The results of this analysis for each motor unit are shown in Figure 4B. Phase-locking of motor units to EEG was greatest for frequencies approaching 20 Hz. Overall, phase-locking between motor units and EEG activity is a time-varying effect. To determine if episodes of SMU-EEG phase-locking corresponded to fluctuations in EEG power at a given frequency, we calculated a Spearman correlation coefficient between SMU phase-locking strength and EEG power across time, for each motor unit. There was no significant correlation between phase-locking and EEG power, nor were there any discernable trends in this relationship (data not shown).

Discussion

We investigated cortical control of the human hypoglossal motoneuron pool. Cortical oscillatory activity in the 15-40 Hz range weakly entrains whole-muscle activity, and intermittently entrains the spike-timing of individual motor units. The latter finding is of particular importance because it confirms that cortical drive to MNs can be measured directly, rather than through its effects on synchronization between MNs. In
physiological terms, activating MNs at their preferred (“resonance”) frequencies may
serve to maximize the efficiency of neuromuscular communication (Funk and Parkis,
2002; Parkis et al., 2003). This may be an important mechanism for driving motor nuclei
which receive input from multiple sources of synaptic drive, and which may show
diminished synchronization between MNs as a result.

Methodological issues:

Traditional measures of coherence assume stationary signal properties, and reflect
a combination of amplitude and phase covariance between signals. For neural data (e.g.
EEG), the assumption of stationarity is often erroneous. Moreover, amplitude and phase
relationships between signals are not necessarily coupled, and may be better dealt with
separately (Lachaux et al., 1999; Bruns et al., 2000). There is also some debate as to the
most appropriate methods for preprocessing EMG data (Myers and O’Malley, 2003; Yao
et al., 2007; Boonstra, 2010; Christou and Neto, 2010b, 2010a; Halliday and Farmer,
2010; Neto and Christou, 2010), and pooling coherence data obtained from multiple
recordings (Amjad et al., 1997; Kilner et al., 1999; Baker, 2000; Halliday and Rosenberg,
2000). Further, coherence estimates may be influenced by decisions inherent in its
calculation, such as data segmentation (Terry and Griffin, 2008). Last, a major limitation
of traditional coherence measures is their inability to reliably assess effects of cortical
beta/gamma band input on the spiking of individual motor units. This has been shown in
both simulated data and recordings from muscles known to show strong corticomuscular
coherence when using surface EMG signals (Negro and Farina, 2011a, 2011b).
In contrast, the quantification of SMU-EEG phase-locking uses a time-frequency representation of EEG and therefore is not restricted to stationary data. Analysis of motor unit spike-timing does not require any special data segmentation, or pre-processing of the EMG signal beyond extraction of SMU action potentials, and it is unaffected by correlated amplitude fluctuations between signals. However, phase-locking analysis is primarily suited to detect the effects of input oscillations on spike-timing rather than firing rate. Its application here is appropriate given that we found corticomuscular coherence to be restricted to the beta/gamma range, and individual HMN motor units do not fire at rates fast enough to fluctuate at these frequencies.

EEG-EMG coherence:

Although oscillatory cortical input in the beta/gamma band evokes coherent whole-muscle EMG oscillations, the effect is generally weak and varies across trials. This finding is in agreement with previous studies which show more proximal MN pools exhibit weaker beta/gamma band coherence among motor units, possibly due to lower density of direct cortical-spinal projections (Clough et al., 1968; Lemon, 1993; Marsden et al., 1999), and/or greater interference from non-cortical sources of input. The notion that the magnitude of coherence between MN output and a given input (e.g. cortical drive) may be reduced by inputs from other sources has been demonstrated in simulation studies (Negro and Farina, 2011a). This may account for the weak corticomuscular coherence observed here, given the functional and anatomical diversity of inputs onto hypoglossal motoneurons. In addition, time-varying sources of noise and temporally intermittent corticomuscular coupling may negatively influence the magnitude of coherence. Finally,
it may be relevant that intramuscular recordings reflect a relatively localized distribution of MN activity. Viewed from this perspective, the effectiveness of the pooled coherence methodology in identifying corticomuscular coherence may stem from the fact that multiple recordings from each subject were combined, thus forming a more complete representation of whole muscle activity. Additionally, pooled coherence analysis does not consider inter-subject sources of variability, which may also have contributed to the lack of consistent EEG-EMG coherence across recordings. It is possible that more stringent control of task parameters and sampling paradigms could increase the reliability of the corticomuscular coherence recordings. From a practical standpoint and for the purposes of this study however, it appears that typical measures of EEG-EMG coherence are not particularly robust. Even so, there is no clear interpretation of what (weak or strong) corticomuscular coherence within the beta/gamma band means physiologically. Although alteration of cortical beta/gamma band activity by stimulation or disease can result in dysfunctional motor control (Hammond et al., 2007; Pogosyan et al., 2009), it remains to be determined how much of this is due to altered corticomuscular synchronization per se.

SMU-EEG phase-locking:

*In vitro* studies show that individual neurons respond to oscillatory input with increased spiking and fire with greater temporal precision when the input is delivered at the “preferred” frequency of the cell (Hunter et al., 1998; Fellous et al., 2001; Funk and Parkis, 2002; Parkis et al., 2003; van Brederode and Berger, 2008). Interestingly, the optimal frequency band for stimulating hypoglossal motoneurons overlaps with the
beta/gamma band (van Brederode and Berger, 2008) expected to be a component of cortical drive. When a neuron is activated at its resonance frequency, its spiking becomes phase-locked to the input. Such an effect can be studied using SMU to EEG phase-locking analysis. However, it may not be possible to infer the degree of phase-locking from standard coherence analysis, especially when coherence is weak. For example, in a previous study (Laine and Bailey, 2011) there was little evidence of common oscillatory input in the 15-40 Hz frequency range expected of cortical drive (i.e., no MU-MU coherence > 10 Hz during tongue protrusion). The results of the present investigation of EEG-EMG coherence also suggest that EEG oscillations do not strongly/reliably synchronize firing among groups of MNs. In contrast to this, SMU-EEG phase-locking in the 15-40 Hz range was comparatively reliable. While SMU-EEG phase-locking appeared to be a more sensitive measure of corticomuscular coupling than EEG-EMG coherence, a strict comparison between methods would ideally utilize simultaneously recorded multi-unit and SMU activity (to facilitate paired comparisons), as well as more stringent controls/limits imposed on task parameters (e.g., attention, feedback, muscle activation levels). Nonetheless, on the basis of the current findings we propose that SMU-EEG phase-locking is a useful addition to standard techniques, and may be of particular value for investigating cortical drive to motor nuclei such as the HMN, wherein the integration of multiple input sources may serve to reduce high frequency coherence between MNs (Laine and Bailey, 2011; Negro and Farina, 2011a, 2011b; Rice et al., 2011).
The temporal progression of phase-locking may be of particular importance when comparing entrainment of SMUs with entrainment of entire MN pools. For example, it is possible that SMUs are not often phase-locked to cortical input at the same time as each other, which could explain weak EEG-EMG coherence despite significant SMU-EEG phase-locking. We speculate that the episodic nature of phase-locking may relate to time-varying synaptic noise.

Further studies will be required to fully understand the physiological relevance and experimental manipulability of SMU-EEG phase-locking. For example, investigation of how cortical entrainment of SMU activity relates to synchronization between units, how the phenomenon varies across muscles, and which experimental conditions can alter its strength or time-course could greatly further our understanding of corticomuscular communication. For the HMN in particular, it would also be informative to study cortical entrainment of SMU activities during dynamic movement such as speech, and under conditions where premotor drive may be abnormal (e.g. stuttering, Parkinson’s disease, tremor, etc.). Finally, there is some evidence to suggest that corticomuscular coherence is a function not only of descending cortical input, but also afferent feedback (Witham et al., 2011). Further research will be required to distinguish the effects of afferent feedback vs. descending control when considering the origin of corticomuscular synchronization of genioglossus motor units.
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References


Figure Legends.

Figure 1.

Analysis of multi-unit and single motor unit (SMU) synchronization with oscillatory EEG activity. Panel A shows the raw EEG and EMG signals (top two traces) as well as the rectified EMG, calculated as the root-mean-squared (RMS) signal within a 10 ms moving window. Panel B shows an example SMU recording. The EEG and EMG are shown in the top two traces, and the discriminated SMU action potentials are shown below. The inset displays these action potentials enlarged and overlaid. To quantify cortical entrainment of hypoglossal MNs as a group, the EEG signal was band-pass filtered between 1.5 and 150 Hz, and coherence was calculated between it and the rectified EMG signal. The resulting coherence profile is shown in Panel C. The plot indicates that the frequency of greatest correlation between the EMG and EEG signals was near about 20 Hz. The dashed line represents the 95% confidence level for this recording. To quantify cortical entrainment of SMU spike-timing, Rayleigh’s Z statistic was calculated between each frequency component of the EEG signal and the timing of SMU action potentials. An example phase-locking profile is shown in Panel D. Frequencies at which the Rayleigh Z value exceeded the 95% confidence level (dashed line) indicate that action potential timing was significantly phase-locked to EEG activity at that frequency.

Figure 2.

Summary of EMG-EEG coherence and SMU-EEG phase-locking. The left column summarizes the results of analyzing EMG-EEG coherence across 34 recordings from 6 subjects. The right column summarizes the results of analyzing SMU-EEG phase-locking across 63 SMUs from 13 subjects. Left column: Each row of the color plot shown in A represents the coherence profile from an individual multi-unit recording, as shown in Figure 1C. Coherence magnitudes at each frequency have been converted to Fisher’s Z scores and are represented by the color of each pixel. The histogram in B shows, for each frequency, the percent of total recordings which had significant coherence between EEG and EMG signals. A value exceeding the 95% confidence level (dashed line), indicates that significant coherence occurred more often than expected by chance, according to a binomial test. In panel C, data from all recordings was combined to create a pooled coherence profile. Values exceeding the 95% confidence level (dashed line) indicate significant levels of coherence. Although the data summaries in panels B and C indicate above-chance coherence at frequencies near about 20 Hz, it is clear from panel A that individual recordings do not reliably show discernable peaks in this range. Right column: Each row of the color plot in D represents the phase-locking profile of a SMU, as shown in Figure 1D. The color of each pixel represents the degree of SMU phase-locking (Rayleigh Z value) to EEG. The histogram in panel E displays the percent of motor units which showed statistically significant levels of phase-locking at each frequency. A percent in excess of the 95% confidence level (dashed line) indicates that more motor units were phase-locked to EEG at that frequency than expected by chance,
according to a binomial test. Panel F shows the average Rayleigh Z value for each frequency, taken across the population of recorded units. A 95% confidence level (dashed line) was derived by comparing the average Z values obtained for each frequency with 1000 simulated averages (see methods). The summarized data in panels E and F show strong effects from about 15-40 Hz, and both methods appear to capture effects which can be seen consistently within individual recordings, as displayed in panel D.

Figure 3.

Shuffle test of SMU-EEG phase-locking. Each row of the top panel displays the probability (color) that the phase-locking data observed across SMU recordings could have occurred by chance at each frequency (columns). In this analysis, chance level was derived by shuffling the spike times within each SMU recording, calculating the SMU-EEG phase-locking profile for each spike train, and then testing the results against the experimentally observed data at each frequency using a paired t-test. The lower trace displays the results of collapsing the 30 shuffle test results into a single statistic using Stouffer’s Z-score method. The horizontal line represents the 95% confidence level. Phase-locking was significantly stronger for real data vs. shuffled data across the ~15-25 Hz range, while the trend towards above-chance phase-locking at about 35 Hz did not reach statistical significance.

Figure 4.

Temporal progression of SMU-EEG phase-locking. Panel A shows the time progression of phase-locking for an example SMU recording. The color of each pixel represents Rayleigh Z values, and all non-significant values have been set to 0. Rayleigh Z values were calculated within a 5 second window, translated across the recording with a step size of 2.5 seconds. The unit appears to phase-lock with EEG activity in the 20-30 Hz range in short bursts of a few seconds at a time. Panel B shows, for each motor unit, the percent of recording time during which SMU activity was phase-locked to EEG at each frequency. On the reduced time scale of 5 second epochs, intermittent phase-locking near 20 Hz is the most consistent EEG-SMU phase relationship across the motor unit population.
Figure 1

A

B

C

D

Coherence

Rayleigh Z

Frequency (Hz)

Frequency (Hz)
Figure 2
Figure 3