Title: 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 \( \sim 10 \) 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 \textit{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 \textit{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 \textit{in vivo}, and in this case, provides a more robust measure of cortical drive than entrainment of multi-unit EMG activity.

\section*{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 (Cambride 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 ($Z=\tanh(VC)$, 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 straight
forward 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
de-compose 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
\[e^{i2\pi ft} * e^{-t^2/(2\sigma^2)}\], where t=time, f=frequency (from 1 to 100 Hz), and where the “width” of
each wavelet was constrained to 3 cycles by setting 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
$RZ(f) = N \times \left( \frac{1}{N} \sum_{k=1}^{N} \frac{W_{(k,f)}}{|W_{(k,f)}|} \right)^2$

where $N$ is the number of action potentials used in the analysis and $k$ is 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 $RZ = N \times PLF^2$ (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 $\geq 3$ 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.