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COORDINATION BETWEEN MUSCLES AND THE SENSORIMOTOR CORTEX DURING OROFACIAL BEHAVIORS IN NON-HUMAN PRIMATES

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DEDICATION

In loving memory of Suguna Ramadas Rachakonda and Radhakrishnan Ramachandraiyer. Thank you for challenging me to be better than the best.

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ABSTRACT

Many researchers have posited that mammalian motor patterns are conserved during mastication (Hiiemae 1978; Bramble and Wake 1985; Weijs 1994). While Weijs (1994) was not the first researcher to recognize that transverse jaw movement during jaw closing is produced by asymmetric activation of the superficial masseter, medial pterygoid, and posterior temporalis (Herring 1973 1976 1979; Gorniak 1977 1985; Weijs and Dantuma 1980), he did reify the triplet motor pattern into an ancestral motor pattern modified by natural selection to produce the range of motor patterns observed in extant mammals. However, there is little evidence to suggest that masticatory motor patterns are homologous. Computational studies of a variety of tasks suggest that movement primitives (simple spatiotemporal elements or building blocks) of behaviors, such as mastication, may represent local optima emerging from a learning process, whereby functional criteria are applied to a range of possible ways of moving until a local functional optimum is reached (Flash and Hogan 1985). The present study begins to determine why some motor patterns are more common than others from both a biomechanical perspective and a neural one. The overarching goal was to facilitate information transfer between the fields of biomechanics, neuroscience, and dynamical systems. Specific aim I was to determine the extent to which the triplet motor pattern is conserved during mastication. Specific aim II was to determine whether the CNS treats the triplet motor pattern as a unit of control. Concise and quantifiable definitions for unison, synchrony, and coordination are suggested to facilitate the flow of information between the fields of neuroscience, biomechanics, and dynamical systems. Specific aim III was to determine how functional groups of muscles are organized within the cortex.

INTRODUCTION: ARE MOTOR PATTERNS CONSERVED DURING RHYTHMIC MASTICATION?

Motor patterns are specific sequences of muscle activity (timing and amplitude) directed at producing a specific extrinsic goal (Lauder and Shaffer 1998). Many researchers have posited that masticatory motor patterns are broadly similar across mammals (Hiiemae 1978; Bramble and Wake 1985; Weijs 1994). In this review, we will discuss whether a motor pattern is a character that can be affected by natural selection. In the literature, four explicit hypotheses have been used to explain why mammalian masticatory motor patterns are conserved. Each of these hypotheses is not mutually exclusive. We will discuss the neural, behavioral, and evolutionary implications of these hypotheses and evaluate their validity.

Hypothesis 1: Mammals share masticatory motor patterns because the motor patterns are homologous. There was an ancestral motor pattern and all other mammalian motor patterns have evolved from it.

Hypothesis 2: Feeding motor patterns are conserved among mammals because both the task and the selection pressures acting on it are conserved. Common selection pressures are necessary and sufficient to produce uniformity. Homology is not necessary.

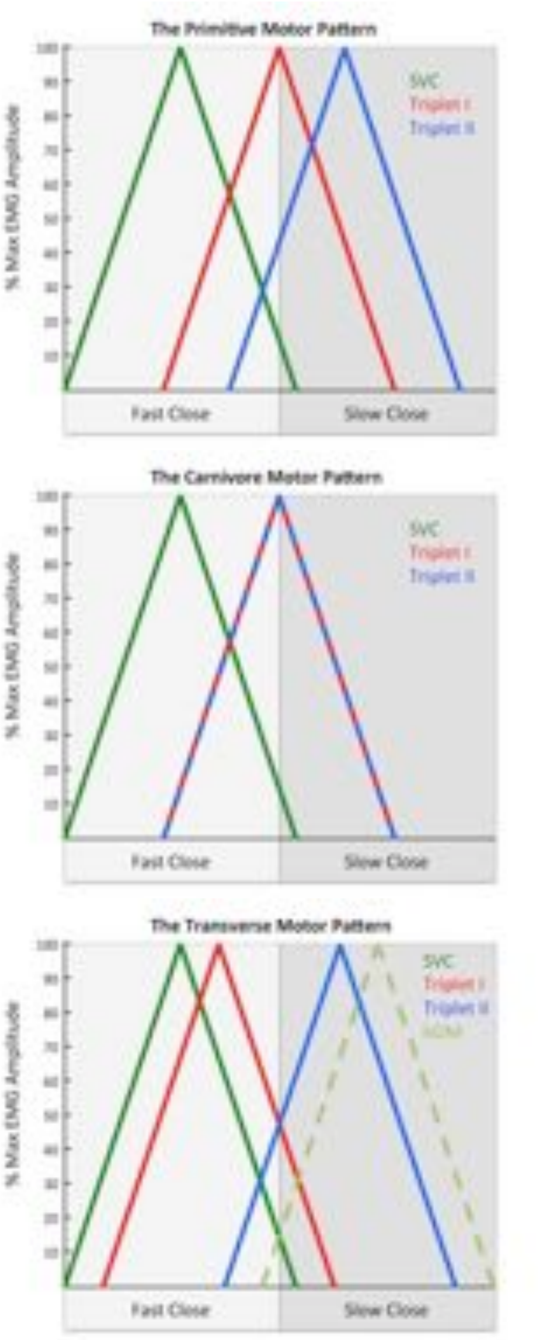
Hypothesis 3: Uniformity in motor patterns is a consequence of the process of masticatory circuit development and ontogeny.

Hypothesis 4: Uniformity in motor patterns is a consequence of functional organization in the sensorimotor cortex.

Hypothesis 1: Motor patterns are conserved because they are homologous

“Homology is the relationship of two characters that have descended, usually with divergence, from a common ancestral character” (Fitch 2000, 1). In order for two characters to be homologous, they must have the same basic structure, the same connectivity, and they must have the same developmental history (Hall 2012, 22). A number of researchers have suggested that there is a primitive mammalian feeding motor pattern that has been modified by the process of natural selection (Bramble and Wake 1985; Weijs 1994). In this section, we will review these claims and use experimental evidence to demonstrate that mammalian masticatory motor patterns are not necessarily homologous.

The hypothesis that motor patterns are homologous developed as an amalgamation of assumptions made by different researchers. Hiiemae (1978) suggested that because motor patterns are broadly similar during mammalian mastication, the masticatory motor pattern is “programmed” within the central nervous system of mammals. Bramble and Wake (1985) furthered this idea by arguing that tetrapods have conserved kinematics, conserved motor patterns, and they utilize homologous muscles. They hypothesized that there was an ancestral feeding motor pattern from which all mammalian masticatory motor patterns have evolved (i.e. mammalian and reptilian motor patterns are homologous) (Bramble and Wake 1985). They argued that if this is the case, then motor patterns may act as a “constraint on evolution” (Smith 1994 294; Bramble and Wake 1985). Liem argued that conserved neuromotor “software” has led to diversification and specialization of “hardware” including teeth, skulls, and jaws (Liem 1984). Thus the hypothesis that motor patterns are conserved grew organically from ideas contributed by various workers in the field.



Weijs (1994) extended the implication that the masticatory motor pattern is a phenotype that can be acted upon by natural selection and evolution. Reviewing pre-existing experimental studies, he presented a hypothesis to describe how various mammalian specializations arose (figure 1). He suggested that for each species, a limited set of muscle activation patterns is represented within the brainstem. The motor pattern activated during an individual cycle determines the kinematics for that cycle. Weijs proposed a specific ancestral motor pattern and adaptations to that motor pattern in extant mammalian clades.

However, none of the workers

Figure 1.1 Weijs (1994) hypothesized that the primitive jaw adduction motor pattern consisted of three distinct muscle groups: symmetric vertical closers (SVC), triplet I, and triplet II. The SVC consists of balancing side (bs) deep masseter (bDM), working side (ws) deep masseter (wDM), bs anterior temporalis (bAT), and ws anterior temporalis (wAT). Triplet I includes the bs superficial masseter (bSM), bs medial pterygoid (bMP), and ws posterior temporalis (wPT). Triplet II includes of wSM, wMP, and bPT. Weijs (1994) hypothesized that the primitive jaw adduction motor pattern was modified in different clades of extant mammals. (a) the primitive motor pattern (b) the carnivore motor pattern (c) the transverse motor pattern (present in ungulates).

described above presented direct evidence to support the claim that mammalian motor patterns are homologous. Smith (1994) observed that the central pattern generator (CPG) plays an essential role in the argument that feeding motor patterns are homologous (Smith 1994). CPGs are small networks of neurons whose activity can generate specific sequences of movement with correct timing in the absence of sensory feedback (Moore et al. 2014). The theory that motor patterns are homologous infers that the motor pattern(s) produced by this CPG are the phenotype affected by natural selection. The mammalian masticatory CPG is located in the brainstem between the rostral portion of the Vth nucleus and the caudal portion of the VIIth nucleus (Tanaka 1999). The neural mechanism that produces the motor patterns and masticatory rhythm in mammals is currently unknown. Neurons of the masticatory CPG must directly connect to the trigeminal (V), facial (VII), and hypoglossal motor nuclei (XII). It must also be directly connected to the parvocellular reticular nucleus, the rostral subdivision of the trigeminal spinal nucleus, and the dorsal portion of the principal trigeminal nucleus (Lund and Kolta 2006). Neurons in these areas fire during either jaw opening, jaw closing, or in between gape phases. In the principal trigeminal nucleus, neurons fire tonically and receive inputs from cells in primary orofacial motor cortex. These neurons fire rhythmic bursts that do not coincide with cortical firing, however some action potentials do occur at a fixed latency after cortical pulses (Lund and Kolta 2006; Tsuboi et al. 2003). Similar to the CPGs for locomotion and respiration, the two hemispheres of the brainstem can produce unilateral fictive mastication (Nozaki et al. 1986). Interneurons in the primary motor nuclei coordinate the masticatory rhythm produced by each “hemi-CPG” (Lund and Kolta 2006). Although the neurons involved in modulating the masticatory motor pattern have been identified, the mechanism for recruiting and modulating specific motor patterns has yet to be determined. Studies have demonstrated that the rhythm and the motor pattern output by the respiratory CPG can be genetically modified by natural selection (Smith et al. 1991; Viemari et al. 2004). However, the masticatory CPG has not been sufficiently studied to determine whether the same holds true.

In sum, many researchers have claimed that masticatory motor patterns are conserved among mammals because they are homologous. However, there is no direct evidence that motor patterns are a phenotype that demonstrates evolutionary and developmental autonomy. It has been demonstrated that rhythmic behaviors are affected by gene regulation of CPG neurons in the brainstem (Viemari et al. 2004) but specific genes that affect the masticatory motor pattern have yet to be identified. While it is possible for the mammalian masticatory motor patterns to be homologous, there is insufficient direct evidence to corroborate or falsify this hypothesis.

Hypothesis 2: Do motor patterns converge when the functional demands on behavior converge?

The second hypothesis argues that common selection pressures are necessary and sufficient for motor patterns to be conserved. Motor patterns do not necessarily need to be homologous to be similar. In this section, I argue that motor patterns converge when the functional demands on behavior converge. The functional demands on behavior include both anatomy and the optimality criteria/ selection pressures acting on the morphology.

Smith (1994) suggested three alternative hypotheses to homology that would explain why masticatory motor patterns are conserved among mammals: (1) that only one basic pattern of mastication is physically possible; (2) that motor patterns are conserved because the behavior is conserved; and (3) that mammalian motor patterns have converged because the functional demands of mastication have also converged. This third hypothesis is not mutually exclusive of the first two.

The hypothesis that only one basic motor pattern is observed in mammals due to morphological constraints is not supported by experimental studies showing that the morphological constraints of mastication change within mammalian clades and so do masticatory motor patterns. For example, in primates, the morphological adaptation of symphyseal fusion is correlated with late activity of the balancing side deep masseter and increased balancing side muscle recruitment (Hylander et al. 2000). Not only is there variation within the mammalian clade, there is variation in the motor pattern within individuals. Vinyard et al. (2008) showed that the greatest variation in motor patterns exists within individuals and within the same sequence. Moreover, the muscles of mastication are redundant; multiple motor patterns will produce the same kinematics. Therefore, conserved mammalian masticatory morphology does not physically constrain the system to one basic masticatory motor pattern.

Smith's second hypothesis states that if the task is unchanged, then the motor pattern will also be unchanged. This hypothesis is supported by studies in salamanders that show that after metamorphosis, only muscles with conserved function have conserved motor patterns (Shaffer and Lauder 1988). However, for motor patterns to be unchanged there must be selection pressure(s) that keep the motor pattern in place, otherwise there would be natural drift. Thus, this hypothesis does not fully explain why motor patterns are conserved.

Smith's third hypothesis suggested that when the functional demands on behavior are conserved, then the motor patterns used to accomplish that behavior are also conserved. Functional demands on a task are conserved when both morphology and the selection pressures on the task are conserved. Experimental studies have shown that when both morphology and the selection pressures acting on a task converge, the motor pattern also converges.

Crompton's 2010 study supports Smith third hypothesis that when the functional demands on a task converge, the motor pattern also converges. Crompton (2010) argued that in marsupials, jaw closing consists of a vertical stroke followed by a medial stroke, with the medial stroke produced by late activity of the working side medial pterygoid and superficial masseter. Crompton (2010) points out that in addition to a modified motor program, marsupials also have an unfused symphysis, inflected angle of the mandible, and an enlarged superficial medial pterygoid. This causes the medial pterygoid of marsupials to have a strong medial line of action that enhances the medially directed kinematics during jaw elevation (Crompton 2010). The basic marsupial condition is exemplified by kangaroos while the sister group to kangaroos, koalas + wombats has derived conditions (Crompton 2010). Like kangaroos, wombats also have a vertical stroke followed by a medial stroke created by the late activity of the working side superficial masseter and medial pterygoid. However, in wombats the balancing side muscles do not fire at all during jaw closing (Crompton 2010). Meanwhile, koalas, the sister group of wombats have a fused symphysis, increased mandibular angle, and a greatly reduced medial pterygoid, similar to placental ungulates. Their jaw elevator motor pattern also resembles that of ungulates, with triplet I firing before triplet II although they were not shown to follow the triplet motor pattern (Crompton 2010). Thus, Crompton (2010) demonstrates that when morphology converges, the motor patterns also converge. This lends support to Smith's (1994) third hypothesis that the motor patterns converge when the functional demands on behavior converge. When morphology converges, there must be a set of selection pressures or optimality criteria that make particular motor patterns more favorable than others.

Hylander et al. (2005) demonstrated that when the task diverges, motor patterns also diverge. Hylander et al. tested the hypothesis that the evolution of symphyseal fusion in primates is associated with a transition from Weijs's (1994) predicted primitive motor pattern to his predicted transverse pattern of mastication. Hylander et al. found that anthropoids but not strepsirrhines show early firing of the WDM and late firing of the BDM. Evolution of symphyseal fusion is correlated with increased wishboning of the symphyses in anthropoids (Hylander et al. 2005). Once again, these findings show that when the functional demands on behavior change, motor patterns also change.

Vinyard et al. (2008) hypothesized that the functional demands of mastication change within a single chewing sequence as the bolus material properties and bite position change. Vinyard et al. looked at significant variation in peak EMG timing and activity (normalized across experiment) at each hierarchical level (i.e. between species, among individuals, within an individual, between sequences, and among chew cycles within a sequence). They found that the greatest variation in peak timing consistently occurred between individual cycles of the same sequence. Vinyard et al.'s (2008) study demonstrates the large degree of variation in motor patterns within a single sequence relative to other organizational levels. They also noted that there are large differences in variance components between the jaw closing muscles. The different variance components across muscles suggest that functional groups like Weijs's (1994) triplets did not evolve as single functional units used by the central nervous system to decrease dimensionality in motor commands (Overduin 2014).

In sum, the studies reviewed in this section demonstrate: (1) When the task (and presumably selection pressures) acting on the task converge, so does the motor pattern; and (2) when the task and selection pressures acting on the task diverge, motor patterns also diverge. Therefore, there is variation in motor patterns within individuals.

Ultimately, functional selection pressures drive variation in feeding motor patterns. The process towards local functional optima is driven by natural selection but local functional optima may not always be realized due to tradeoffs between different optimality criteria (Ross and Iriarte-Diaz 2014). Ross and Iriarte-Diaz (2014) argue that there is strong covariation between "skull morphology and the ability to generate and transmit force" (Ross and Iriarte-Diaz 2014, 10). However the ability to have a large gape affects force production (Hylander 2013). Rostral displacement of jaw closing muscles to improve mechanical advantage and increase bite force reduces the maximum possible gape (Ross and Iriarte-Diaz 2014). Effective chewing requires that compressive forces are maintained on the TMJ. To accomplish this task during unilateral (non-midline) chewing, the balancing side muscle force must be reduced (Ross and Iriarte-Diaz 2014). Thus, the motor patterns observed in mammals represent local functional optima that result from tradeoffs between multiple selection pressures. Natural selection is one potential mechanism that drives motor patterns towards a set of local functional optima. However, motor patterns are also pliable within individual lifetimes.

Theory 3: Are conserved motor patterns a consequence of the developmental process?

During development, anatomical and physiological changes in muscle architecture and anatomy go hand in hand with increased neural control and reorganization of neural circuits to meet the functional/behavioral demands of each developmental stage (Herring 1985). One key constraint on the system is that it must be functional throughout development. Therefore, developmental processes influence the range of possible masticatory patterns.

Herring demonstrated that the transition from suckling to chewing is gradual but the events appear in a stereotyped order. Part of this transition is preprogrammed but there is substantial learning. This is evidenced by the fact that premature mastication does not resemble mature mastication (Westneat and Hall 1992; Langenbach 2001). The development of occlusion is correlated with the emergence of a premature masticatory motor pattern that closely resembles suckling (Westneat and Hall 1992). The motor pattern observed during this developmental phase appears to be an intermediate between suckling and adult mastication (Langenbach 2001). Occlusion and sensory feedback from the periodontal afferents are also argued to play a key role in the transition from the immature masticatory motor pattern to the mature masticatory motor pattern (Langenbach 2001). However, development of occlusion is not necessary to induce the shift from suckling to chewing (Iriki et al. 1988; Ochiai et al. 1961). Mammals born with complete dentitions still suckle during the first week and undergo the transition from suckling to mastication postnatally (Ainamo 1971). In all mammals, individual specific behaviors become ingrained during this transition from suckling to chewing (Langenbach 2001). These ideas are novel because they suggest that there is reciprocal causality between morphology and behavior during masticatory development.

Studies have shown that the shift from suckling to mastication is associated with a reorganization of the masticatory circuit. In adult mammals, stimulation of the cortical masticatory area (CMA) induces rhythmic mastication (Luschei and Goldberg 2011; Nozaki et al. 1986). The CMA projects to the paragigantocellular reticular nucleus via the pyramidal tract and the paragigantocellular reticular nucleus is connected to the principal sensory nucleus of the trigeminal nerve (V) where the CPG for mastication is thought to be located (Nozaki et al. 1986). Iriki et al. (1988) demonstrated that the cortical sucking area (CSA) is located rostral to the cortical masticatory area. In neonatal (1 day old) guinea pigs, stimulation of the CSA produces rhythmic jaw behavior while stimulation of CMA does not. The reverse is true in adult mice (3 months old). In adult mice, stimulation of the CMA but not the CSA produces rhythmic jaw movements. Irki et al. suggest that in neonates, the CSA not the CMA innervates the paragigantocellular reticular nucleus. Iriki et al. hypothesized that the transition from suckling to mastication is associated with a reorganization of the masticatory circuit.

It is currently unknown how reorganization of the masticatory neural circuit impacts the range of possible masticatory motor patterns. Reciprocal causality could also be true: i.e., changes in behavior aided by plasticity in the CNS could trigger reorganization of the neural circuit. Plasticity in the CNS is maintained throughout ontogeny and the physical factors affecting behavior can change during ontogeny. Therefore, the range and frequency of motor patterns utilized by an individual could potentially change throughout ontogeny, not just during the transition from suckling to chewing.

Theory 4: Is motor pattern conservation associated with functional organization of the sensorimotor cortex?

Researchers have suggested that the functional organization of the cortex contributes to the conservation of specific motor patterns (Overduin et al. 2014). Complex movements like mastication can be broken down into simple spatiotemporal elements or building blocks called movement primitives (Wolpert et al. 2000). Computational studies of a variety of tasks suggest that these movement primitives represent local functional optima as discussed by Ross and Iriarte-Diaz (2014) for the masticatory system and Flash and Hogan (1985) for reach and grasp. A set of functional criteria are used to assess all possible ways of moving and there is gradual improvement until a local functional optimum is reached (Flash and Hogan 1985). This implies that a repertoire of motor primitives and/or muscle activation patterns is stored somewhere in the CNS (Wolpert et al. 2000). This repertoire must be malleable to both natural selection and plasticity during ontogeny (Wolpert et al. 2000). However, the causality between stereotypy in behavior and functional organization in the sensorimotor cortex is currently under debate.

Graziano and colleagues have suggested that the cortex contains distinct functional zones. Stimulation of these zones produces ethologically relevant actions. They suggested that cortical representation is influenced both by the structure of the body and common actions in the behavioral repertoire (Graziano 2016). Based on this theory, each zone in the cortex represents an ethologically relevant behavior like chewing, lapping, or reach to grasp. Electrical stimulation anywhere in this cortical zone leads to a variant of the underlying behavior (Graziano 2016). Graziano (2002) stimulated different parts of the cortex for half a second, a duration which is on the same time scale as natural movement. This resulted in complex movements like mastication and reach to grasp. The elicited behaviors were arranged somatotopically across both the motor and premotor cortex. However, variations of each movement were clustered together within the larger somatotopic gradient (Graziano 2002).

Studies in the orofacial primary motor cortex support the hypothesis that behaviors are organized ethologically within the sensorimotor cortex. Various masticatory patterns are represented separately in the motor cortex (Lund et al. 1984). Stimulation of the anterior and posterior regions in rabbits produces distinct masticatory patterns. Stimulation of the right and left cortices produces masticatory movements that are mirror images of each other (Lund et al. 1984; Martin et al. 1997; Yao et al. 2002; Lund and Kolta 2006). Moreover, high frequency stimulation of M1 (via intracortical microstimulation) produces discrete orofacial behaviors like lapping and swallowing while low frequency stimulation of S1 produces rhythmic behaviors like mastication and lapping (Huang et al. 1989a; Martin et al. 1999; Yao et al. 2002).

Bizzi, Mussa-Ivaldi, Gizster and, others have proposed that all movement is composed of simple modular building blocks called muscle synergies (Bizzi et al. 1991; Mussa-Ivaldi et al. 1994). A muscle synergy is a group of functionally related muscles that are activated with stereotyped amplitude and timing. The muscle synergy hypothesis states that muscle synergies are stored within the CNS and that all movement is produced by the linear combination of muscle synergies (Alessandro et al. 2013). However, this hypothesis has been difficult to prove or falsify (Kutch and Valero-Cuevas 2012). Low frequency stimulation of M1 produces discrete orofacial behaviors like lapping and swallowing while high frequency stimulation of S1 produces rhythmic behaviors like mastication and lapping (Huang et al. 1989a; Martin et al. 1999; Yao et al. 2002). Recordings from specific intracortical electrodes have also shown that some electrodes have a high spike rate when specific muscles are weakly active but not strongly active (Schieber 2001). Muscle synergies have been proposed as a solution to an "ill posed" problem (Mussa-Ivaldi et al. 1990). For any given movement, there are a large number of muscle activation strategies that would produce the desired kinematics (Mussa-Ivaldi et al. 1990). According to Bizzi and colleagues, muscle synergies reduce dimensionality within the CNS. If muscle synergies act as constraints on movement, then they should also be able to explain suboptimal performance (Tresch and Jarc 2009). Instead, "others have suggested that muscle synergies provide a way for the CNS to bootstrap complex problems of optimal control" in dimensions that are not task relevant (Tresch and Jarc 2009, 1).

The uncontrolled manifold hypothesis suggests that the CNS identifies and optimizes only task relevant variables. "Variability which does not affect the task can be allowed without penalty since attempts to correct such task irrelevant variability would be an unnecessary waste of effort" (Tresch and Jarc 2009, 5). Based on this hypothesis, variability during mastication reflects efficient control. Here, the task related variables represent the optimality criteria or selection pressures discussed in the section labeled theory two.

From the neural and control perspectives, muscle synergies and motor primitives can serve one of two functions. (1) They may be a constraint on movement control, or (2) they may be an optimization strategy (Nazarpour et al. 2012). The first case implies that motor patterns are conserved due to hard-wired neural circuitry. The second scenario implies that because any movement of a body part is influenced by similar biomechanical principles, the optimal muscle synergies and/or motor primitives will be broadly conserved (Nazarpour et al. 2012). If conservation in motor patterns is a result of optimization, then any repertoire of muscle synergies and /or motor primitives stored within the sensorimotor cortex would serve as a reserve of optimal shortcuts as oppose to a neural constraint on behavior.

SPECIFIC AIMS

Aim 1: Are motor patterns conserved during rhythmic mastication in primates?

Previous workers have used the mean onset, peak, and offset times of triplet I and triplet II muscles to determine whether specific species follow the triplet motor pattern. However, workers in the field often utilize either experiment means (Williams et al. 2007) or each individual's mean (Hylander et al. 2005) to calculate the species mean and standard deviation. These techniques obscure the variation in motor patterns within individuals and between individuals of the same species. Therefore, Aim 1 assesses the prevalence of the triplet motor pattern in each of five primate species: *Eulemur fulvus, Propithecus verreauxi*, *Papio anubis*, *Macaca fuscata* and *Pan troglodytes*. The findings of Aim 1 suggest that there is flexibility in muscle activation patterns indicating that the jaw elevator motor pattern is not a fixed binomial trait. There is variability between cycles and the motor pattern can be modified between onset, peak and offset.

Aim 2: Do the triplet muscles represent a unit of motor control? Are they a muscle synergy?

Aim 2 utilizes phase information and cyclic statistics to quantify the interaction between jaw elevator muscles. Specific and quantitative definitions of unison, synchrony, and coordination are proposed and used to investigate the role of the CNS in modulating motor patterns to produce goal oriented force production and kinematics. The following hypotheses are tested: muscles in the same triplet are more in unison than muscles in two different triplets; activity of muscles in the same triplet is more tightly synchronized than activity of muscles in two different triplets; and muscles in the same triplet are more closely coordinated than muscles in two different triplets. The findings of Aim 2 suggest that triplet I is more tightly modulated by the CNS than triplet II. The triplet motor pattern may be functionally significant earlier in the masticatory cycle, but as the mandible comes in contact with the bolus during slow close, the motor pattern is modified based on sensory feedback. Thus, the triplet motor pattern is not maintained throughout the cycle. Moreover, a different pattern of coordination spears to be used during non-triplet cycles, leading to increased coordination between non-triplet muscle pairs and decreased coordination between triplet muscle pairs. Further studies are needed to understand the full range of jaw elevator motor patterns and their relationship to jaw kinematic and the stages of the chewing sequence. Ultimately, the goal is to identify the importance of different optimality criteria in the feeding system (Ross and Iriarte-Diaz 2014) and the neural factors that may influence specific muscle activation patterns (Mussa-Ivaldi et al. 1990).

Aim 3: How are functional groups of muscles organized in the primary orofacial cortex (MIo)? Are specific functional groups of muscles clustered together on the cortical sheet?

MIo is located in the most lateral region of primary motor cortex (MI). Huang et al. (1988) demonstrated that representations of the face, jaw, and tongue are organized medial to lateral (in that order) with a large region of overlap between the three areas. However Graziano et al. (2002) demonstrated that the cortex contains functional zones, which represent ethologically relevant actions. Graziano et al. (2002) also demonstrated that variations of each movement are clustered together within the larger somatotopic gradient. Aim 3 utilizes an ICMS protocol developed by Park et al. (2001) to determine whether functional groups are spatially clustered together within MIo. The ICMS protocol used (Park et al. 2001) is specific and nondisruptive to minimize current spread. The findings of Aim 3 suggest that muscles that act synergistically are positively correlated. Jaw depressors, hyoid depressors, and tongue protruders are all often correlated together. However antagonists are also often recruited together possibly to produce asymmetric movements. Additionally, we found that the MIo of two macaques was organized into distinct spatial clusters that represent functionally relevant behaviors. The MIo of both monkeys was organized anteromedially to posterolaterally (diagonally) as oppose to medial to lateral. Moreover, the monkey that was trained to produce precision forces during biting regularly for four months continuously had distinct zones in MIo that represent jaw elevation while the second monkey which was trained for half that time did not. Previous researchers found that jaw depression was represented in MIo however jaw elevation was evoked in highly limited locations. The findings of the present study suggest that spatial clustering within MIo and the functional behaviors that are represented by these clusters may potentially be modified in a use dependent manner.

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EVALUATING THE TRIPLET HYPOTHESIS DURING RHYTHMIC MASTICATION IN PRIMATES

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ABSTRACT

Mammalian mastication involves precise jaw movements including transverse movement of the mandible during the power stroke. Jaw elevation and transverse movement are driven by asymmetrical jaw elevator muscle activity which is thought to include a phylogenetically primitive and conserved *triplet motor pattern* consisting of: triplet I—balancing side superficial masseter, medial pterygoid, and working side posterior temporalis— which reaches onset, peak, and offset first; and triplet II—working side superficial masseter and medial pterygoid, and balancing side posterior temporalis—which is active second. Although the presence of a triplet motor pattern has been confirmed in several primate species, the *prevalence* of this motor pattern—the proportion of cycles that display this pattern—has not been evaluated in primates. The present study quantifies the presence and prevalence of the triplet motor pattern in five different primate species, *Eulemur fulvus*, *Propithecus verreauxi*, *Papio anubis*, *Macaca fascicularis*, and *Pan troglodytes*, using mean onset, peak, and offset time relative to working superficial masseter. In all five of the species studied, the mean triplet motor pattern is observed at peak muscle activation, and in four out of the five species the triplet motor pattern occurs more frequently than expected at random at peak muscle activation and offset. Non-triplet motor patterns were observed in varying proportions at different time points in the cycle, suggesting that presence or absence of the triplet motor pattern is not a binomial trait. Instead, the primate masticatory motor pattern is malleable within individual cycles, within individual animals, and therefore within species.

INTRODUCTION

Table 2.1. Muscle Abbreviations

Muscle	**Abrev**	**Working Side**	**Balancing Side**
Superficial Masseter	SM	wSM	bSM
Deep Masseter	DM	wDM	bDM
Masseter	M	wM	bM
Medial Pterygoid	MP	wMP	bMP
Anterior Temporalis	AT	wAT	bAT
Posterior Temporalis	PT	wPT	bPT
Temporalis	T	wT	bT

Table 2.1 Muscle abbreviations used for the jaw adductor muscles studied in this paper.

Motor patterns are cyclic "repeating sequence(s) of motor neuron activity produced during an actual or fictive motor act" (Binder et al., 2009). Cyclic chewing behavior is characterized primitively—and in many extant mammals—by precise occlusion, unilateral chewing, high levels of rhythmicity (Ross et al., 2010), and lateral-to-medial tooth and jaw movements on the biting side during the slow close phase (power stroke) of the jaw gape cycle (Hiiemae, 1976; Williams et al., 2011). The transverse movements during slow close are often ascribed to a specific *motor pattern* characterized by asymmetric activation (both in amplitude and timing) of the bilateral jaw elevator muscles—masseters, temporales and medial pterygoids (Herring, 1973, 1976, 1979; Gorniak, 1977, 1985; Weijs and Dantuma, 1980). Weijs (1994) reified the triplet motor pattern into an ancestral motor pattern modified by natural selection to produce the range of motor patterns observed in extant mammals (shown in Figure 1).

According to the triplet hypothesis, the lateral-medial working side jaw movements during jaw elevation are produced by a specific activation sequence of the

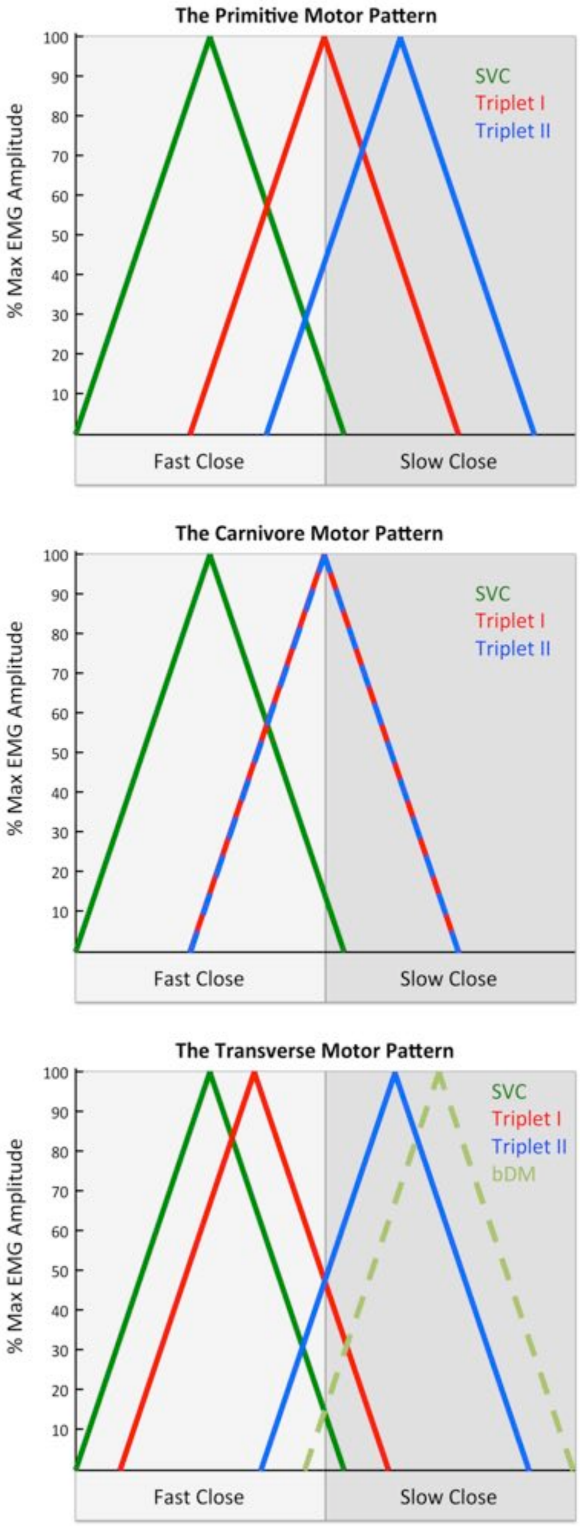


Figure 2.1 Weijs (1994) hypothesized that the primitive jaw adduction motor pattern was modified in different clades of extant mammals. (a) the primitive motor pattern (b) the carnivore motor pattern (c) the transverse motor pattern.

working side (ws) and balancing side (bs) superficial masseters (SM), posterior temporales (PT), and medial pterygoids (MP). In Weijs' primitive motor pattern, the symmetric vertical closers (SVCs)—balancing and working side deep masseters (bDM and wDM) and anterior temporalis (bAT and wAT)—fire first during the chewing cycle, followed by triplet I (bSM, bMP the wPT), which rotates the mandible towards the ws as it is elevated, then triplet II (wSM, wMP, and bPT), which rotates the jaw towards the bs at the end of jaw elevation.

Weijs (1994) hypothesized that this primitive mammalian masticatory motor pattern has been modified differently in different groups of extant mammals. He suggested that in carnivores, the two triplets are active at the same time, producing a predominantly vertical jaw motion, which, in combination with the carnivore dental morphology, produces the vertical occlusal shearing movement mandated by a carnivorous diet. In contrast, ungulates and large herbivores increase the temporal offset between triplets I and II in order to increase transverse movement of the jaw, producing the grinding movement mandated by a herbivorous diet. Weijs hypothesized that in species with a transverse motor pattern the SVCs either fire as a group before both triplet I and II, or the bDM fires after triplet II.

Support for the triplet hypothesis is variable and clade specific (Figure 2). In macropod marsupials an orthal (vertical) phase of jaw closing is accompanied by activity in the bSM and bMP, closely followed by working and balancing AT and PT (Crompton et al., 2008a), then a transverse jaw movement phase is accompanied by activity of wSM and wMP (Crompton et al., 2008a). In wombats, triplet motor patterns are not seen as only the working side jaw elevators are recruited during a completely transverse jaw closing phase (Crompton et al., 2008b). Only koalas display a triplet motor pattern: the wDM reaches peak first, followed closely by wAT in association with triplet I, bAT in association with triplet II, followed by the bDM. In tree shrews (*Tupaia*), small, insectivorous, frugivorous mammals often argued to be the primate sister group, triplets I and II are observed but the SVCs are not all active before triplet I (Vinyard et al., 2005). Instead, the ATs are active with their ipsilateral PTs, the bDM fires in association with triplet I, and the wDM fires in association with triplet II. Williams et al. (2007) found little support for the triplet jaw elevator motor pattern in ungulates. In alpacas, the triplet motor pattern was significant at onset but not at peak or offset, whereas in goats and horses the triplet motor pattern was insignificant at onset, peak, and offset. Thus, the marsupial and ungulate jaw elevator motor patterns provide little support for Weijs' model of triplet motor pattern evolution: a triplet motor pattern is only observed in one marsupial and (partially) one ungulate.

In primates, the triplet motor pattern has been identified in *Sapajus* and *Cebus* (Williams et al., 2011), *Homo* (Moller 1966; Langenbach and Hannam, 1999), *Papio*, and *Macaca,* but not in *Aotus* and *Callithrix* (Hylander and Johnson, 1994; Hylander et al., 2000, 2005; Vinyard et al., 2006). Hylander and colleagues relate variation in the existence of the triplet motor pattern to variation in jaw morphology and kinematics (Hylander and Johnson, 1985, 1994; Hylander et al., 1987, 2000, 2004, 2005). Strepsirrhines, the sister group to tarsiers and anthropoid primates, mostly have unfused mandibular symphyses, which Weijs predicted would be associated with the primitive mammalian motor pattern, and anthropoids (New and Old World monkeys) have fused symphyses, which Weijs predicted would be associated with the transverse motor pattern characteristic of other herbivorous mammals (Weijs, 1994; Hylander and Johnson, 1984; Hylander and Johnson, 1985). In galagos (*Otolemur*), the triplet motor pattern is observed, but the bDM fires with triplet I and the wDM fires with triplet II: the SVCs do not fire before triplet I. Hylander et al. (2000) noted that delayed and increased bDM activity in anthropoids prolongs the power stroke, and increases transverse jaw movements, suggesting that late recruitment of the bDM and lower working/ balancing DM ratios in anthropoids increase transverse components of bite force and contribute to the wish-boning deformation regime of the mandible during the power stroke. They hypothesized that this might be related to the evolution of symphyseal fusion in anthropoid primates, a hypothesis corroborated by the masticatory motor patterns of *Propithecus verreauxi*, a strepsirrhine that gradually develops a partially fused mandibular symphysis after birth, displaying nearly complete symphyseal fusion by adulthood (Hylander et al., 2011). Because *Propithecus* evolved symphyseal fusion independently of anthropoids, Hylander et al. predicted that they would also demonstrate anthropoid-like jaw elevator motor patterns. As predicted, *Propithecus* display increased bDM and bPT muscle activity amplitudes; and firing of DM with the contralateral SM, not the SVCs (Hylander et al., 2011). The lineage leading to extant *Propithecus* also evolved—convergently with anthropoid primates—a fused mandibular symphysis, condyles positioned high above the tooth row, and vertically aligned jaw muscles, supporting the hypothesis that modified motor patterns are correlated with modified morphology and kinematics in primates (Ravosa et al., 2000).

Together these data suggest that there is interspecific variation in the existence of the triplet motor pattern, and that the occurrence of a triplet motor pattern may be related to other aspects of feeding system structure and function (Figure 2). Williams et al. (2011) tested for the concerted evolution of triplet I and II using pre-existing data, treating triplet I and II as binomial traits (i.e., present or absent) and using maximum likelihood and the Bayesian Markov Chain Monte Carlo method to test for correlated evolution across the mammalian phylogeny. Their hypothesis was corroborated by both tests, suggesting that the two triplets evolve together and hence may be acted on by natural selection for their advantage according to some unspecified optimality criterion.

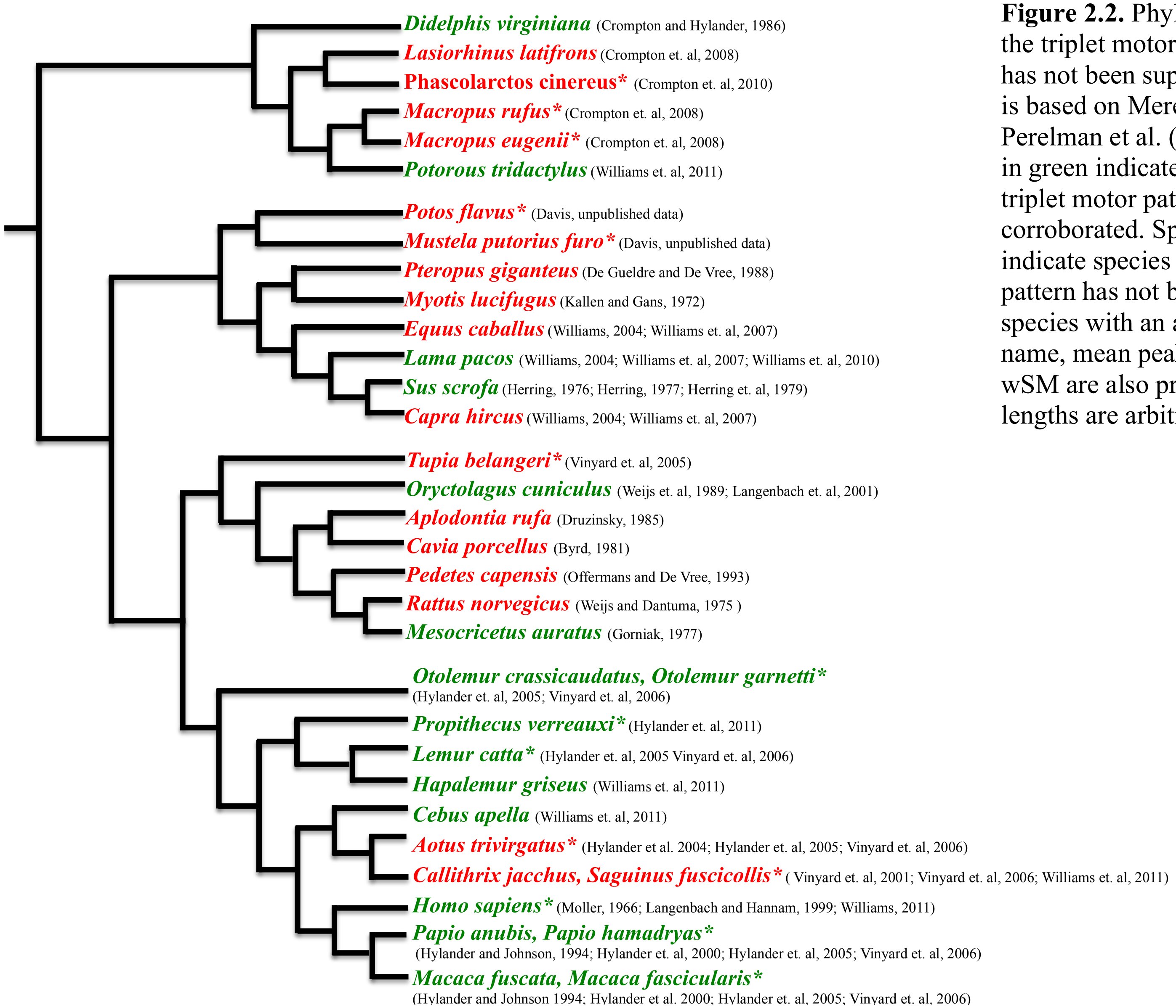


Figure 2.2. Phylogeny of species where the triplet motor pattern either has or has not been supported. The phylogeny is based on Meredith et al. (2011) and Perelman et al. (2011). Species names in green indicate species where the triplet motor pattern has been corroborated. Species names in red indicate species where the triplet motor pattern has not been supported. For species with an asterisk next to their name, mean peak times relative to wSM are also provided. The branch lengths are arbitrary.

Table 2.2. Peak muscle firing time (in milliseconds) relative to wSM and standard deviation (where available)

Species	wDM	wAT	wPT	bSM	bMP	bDM	bAT	bPT	wMP
Phascolarctos cinereus	-2 ± 15	0.5 ± 15	-4.9 ± 15	-44.4± 19	-30.9 ± 20	-41.8 ± 15	-29.6 ± 24	-42.8 ± 15	6.5 ± 18
Macropus rufus		-40 ± 15		-66 ± 17	-74.5 ± 39		-50 ± 26		0.5 ± 14
Macropus eugenii		-35 ± 14		-53.3 ± 25	-70 ± 24		38.3 ± 24		0 ± 18
Potos flavus	-2	2	1.7	0.3	0.3	5	2	1.1	3.4
Mustela putorius furo	-0.3	4.3	3.5	0.1	2.8	0.2	5.7	4.7	4.6
Tupia belangeri	3 ± 5	12 ± 3	-4 ± 2	10 ± 5		8 ± 6	-2 ± 3	12 ± 3	
Otolemur crassicaudatus, Otolemur garnetti		13 ± 21.5	14 ± 14.4				-12 ± 19.4	-3.3 ± 13.2	
Otolemur crassicaudatus, Otolemur garnetti	11			21		23			
Propithecus verreauxi	35 ± 14.9	20 ± 13.1	18 ± 13	15 ± 13.4	16 ± 13.6	-23 ± 11.5	-12 ± 11.9	1 ± 11.9	
Lemur catta		23.1 ± 15.5	17.2 ± 12.9				6.1 ± 14	-0.1 ± 11.8	
Lemur catta	37			14		24			
Aotus trivirgatus		12.9 ± 17.1	13.3 ± 16.3				-13.8 ± 15.6	-15 ± 16.8	
Aotus trivirgatus	13			-1		-11			
Callithrix jacchus, Saguinus fuscicollis	10			-5		-12			
Homo sapiens			-10	-30	-60		20	10	0 ± 10
Papio anubis, Papio hamadryas		16.2	20.4				-6.2 ± 19	-18.9 ± 20.3	
Papio anubis, Papio hamadryas	47			17		-6			
Macaca fuscata, Macaca fascicularis		7.9 ± 15.7	7.1 ± 19.1				-6.4 ± 13.9	-16.3 ± 15.1	
Macaca fuscata, Macaca fascicularis	65			17		-20			

Table 2.2 Times of jaw muscle peak activity for various mammalian species. Negative values indicate that the muscle reached peak after wSM while positive values indicate that the muscle reached peak before wSM. The number following the ± is the standard deviation. Species names in green indicate that the triplet motor pattern is present while species names in red indicate that the triplet motor pattern was not present. Crompton (2008, 2010) provided the median timing for selected chews for each individual. Medians for each individual were summed before taking a grand species mean (presented here). In cases where data were summed, the largest standard deviation is listed. Data from: Møller, 1966; Langenbach and Hannam, 1999; Hylander et al. 2005; Vinyard et al. 2005; Vinyard et al. 2006; Crompton et al. 2008; Crompton et al, 2010; Hylander et al. 2011; Williams 2011; Davis unpublished data.

The literature reviewed above argues for "consistently identifiable patterns of muscular contraction that characterize feeding behaviors both across individuals and among species. These muscle activity patterns, or motor patterns, are characterized by consistent order, duration and/or magnitude of muscle activation during specific feeding tasks" (Williams et al., 2011, 248). In addition to this *inter-specific* variation in the existence of triplet motor patterns, there is also variability in the prevalence of the motor pattern between cycles within individual animals that is rarely quantified but important for understanding the control of mammalian jaw movements. To date, the only paper documenting this variability is Williams et al. (2007) in which a one-tailed binomial test was used to evaluate the hypothesis that the triplet motor pattern is the most common motor pattern at onset of activity, peak activity, and offset of activity. However, by first calculating the experimental mean onset, peak, and offset times, then testing the hypothesis using the experimental means, this method de-emphasizes intra-individual variation within experiments and takes into consideration only the inter-individual variation within a species.

The importance of cycle-to-cycle variation in relative muscle timing is highlighted by studies showing that there is more variation in jaw elevator muscle relative timing and jaw kinematics *within chewing sequences* on a single piece of food than *between chewing sequences on different foods* (Vinyard et al., 2008; Ross et al, 2012; Ross and Iriarte-Diaz, 2014). With this in mind, the present study documents variability in the triplet motor pattern across primates. The existence of a triplet motor pattern in primate species was supported by the *mean* timing of peak muscle activity relative to wSM (Hylander and Johnson, 2000; Hylander et al., 2005; Vinyard et al., 2005). If all the muscles in triplet I (bSM, bMP, wPT) reach *peak* muscle activity before all the muscles in triplet II (wSM, wMP, bPT), then occurrence of the triplet motor pattern is confirmed. However, the evolutionary and functional significance of the triplet motor pattern is also related to the *proportion* of chewing cycles displaying a triplet motor pattern, and whether the muscles show the triplet motor pattern at onset, peak and offset. Calculating the proportion of cycles that follow the triplet motor pattern in each species promises insight into the distribution and variability in jaw elevator motor patterns within primate clades, and will generate the first estimate of the intra-specific prevalence of triplet motor patterns in the well-sampled primate order. Moreover, by looking at three different time points during the gape cycle (onset of muscle activity, peak muscle activity, and offset of muscle activity), this study sheds light on the biomechanical factors influencing the prevalence of the triplet motor pattern, informing hypotheses about the selection pressures establishing and maintaining triplet motor patterns in primate lineages. In sum, understanding when and how frequently the triplet motor pattern occurs will be helpful in understanding not only *if* the triplet motor pattern is more common than other jaw elevator motor patterns but also *why* the triplet motor pattern is so common within the mammalian clade.

Hence, this research asks two questions: *What proportion of chew cycles displays a triplet motor pattern? Do the jaw elevator muscles show consistent patterns of "triplet" behavior at onset, peak, and offset?*

MATERIALS AND METHODS

Data selection

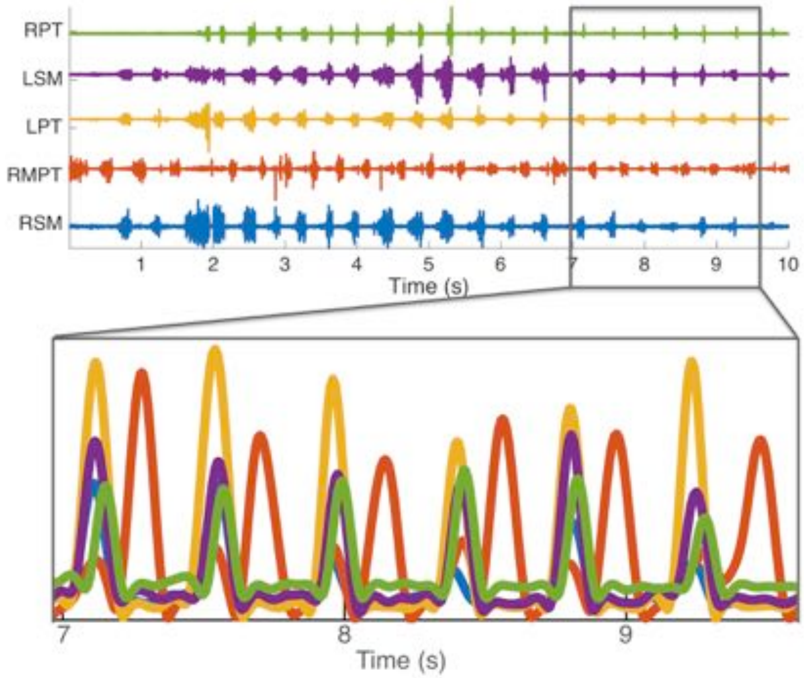


Figure 2.3 Sequence from FEED database that includes MP. The subplot on top shows the raw EMG data for each channel. The bottom subplot shows the EMG after it was filtered (see methods for details). Note that the channel labeled MP has two bouts of activity per cycle. One bout is in phase with the jaw elevators and the other is anti-phase with the jaw elevators. This indicates that there is cross talk between the medial pterygoid and the digastric muscle. For this reason MP was excluded from our study.

Data used in this study are from *Propithecus verreauxi*, *Papio anubis*, and *Macaca fuscata,* which were downloaded from the FEED database (Wall et al., 2011), and from *Eulemur fulvus*, and *Pan troglodytes*, which were extracted from data files previously collected by one of us (CFR). Sequences were selected for analysis if chewing side could be identified and the EMG data included enough triplet muscles and were of good quality (not clipped or too noisy). Sequences from the FEED database that had EMG values for bSM, wSM, bPT, and wPT were utilized in this study. If chewing sequences in the database included two channels of recordings from the same muscle, one of the two signals was arbitrarily chosen based on the following criteria: most constant baseline, least baseline noise, and largest unclipped amplitude during rhythmic mastication.

Chewing sequences for *Macaca, Papio*, and *Propithecus* were labeled in the FEED database as exclusively left or right chews. Chewing side for *Eulemur* was recorded on the voice

Table 2.3. Number of cycles per individual

Species	Individual	Number of cycles
Eulemur fulvus	B	122
	H	118
Propithecus verreauxi	C	57
Papio anubis	M	25
	G	19
	C	16
Macaca fuscata	B	328
	S	241
	T	265
Pan troglodytes	O	87
	A	138

Table 2.3 Number of cycles used in this study per individual

track of the tape recorder or in experimental notes during data collection and corroborated using changes in principal strain orientation recorded from the mandible. For *Pan*, the chew side was determined from the direction of jaw movement during the slow close phase of the gape cycle as seen on videos of the recording session: if the mandible was moving towards the left, then it was a right chew and vice-versa. However, jaw movement was not visible for all cycles, so for the remaining cycles, a clustering algorithm that utilized EMG data for all jaw elevator muscles was used to determine the working side. The clustering algorithm successfully classified all those cycles for which working side could be seen in video and so it was assumed to accurately reconstruct chewing side for the remaining cycles.

Pre-processing

The data were full wave rectified and a 4th order low pass Butterworth filter with cutoff at 30 Hz was applied followed by a root mean square (RMS) moving window integration with a 42 ms integration window. All the data were collected at10 kHz with exception of *Pan* and

Eulemur data (1 kHz). For each sequence all four channels were plotted and the starting point for each cycle manually selected such that all four cycles had minimal EMG activity at the start of the cycle and each channel reached peak amplitude only once during the cycle. Every cycle was manually reviewed to ensure that no channels were clipped and all cycles represented only one complete cycle of jaw closing EMGs. Muscle names were changed to include ws and bs. Individual channel amplitudes were normalized from 0 to 1 within each cycle by subtracting the minimum and dividing by the maximum value. Examination of the integrated and rectified EMG signal for medial pterygoid (MP) from the FEED database revealed EMG activity during jaw depression (Figure 3). This was interpreted as cross-talk between the medial pterygoid and the posterior belly of the digastric muscle: therefore, all medial pterygoid data were excluded from the analysis.

Mean onset, peak, and offset time.

Onset was defined as the last time point before peak amplitude when standardized amplitude is $\leq$ 0.25. Peak amplitude was defined as the time point in the cycle when standardized amplitude equals 1.0. Offset was defined as the first time point after peak amplitude when amplitude is $\leq$ 0.25. The offset could occur up to 8 ms after the end of the cycle. Onset, peak, and offset frames were multiplied by recording frequency to obtain onset, peak, and offset time. Finally, onset, peak, and offset time of working side superficial masseter was subtracted from that of all other muscles to obtain onset, peak, and offset times relative to working side superficial masseter. Times less than 30% of total cycle length were discarded. Offset times less than or equal to 60% of total cycle length were discarded. If both onset and offset times for a given cycle were discarded then the cycle was excluded from all further analyses. Cycles with a peak amplitude time less than onset or greater than offset were also excluded from all further analyses.

The standard technique for determining whether a species follows the triplet motor pattern involves calculating the mean muscle onset, peak and offset times (see Table 1 for a list of studies that have previously used this method). Based on this method, if all the muscles in triplet I have mean onset, peak, and offset times before triplet II, then the species follows the triplet motor pattern. A one-way t-test was used to determine whether the distribution of onset, peak, and offset times was significantly different ($p \leq 0.05$) from a normal distribution around zero (onset, peak, and offset time for wSM) with unknown variance.

Proportion of cycles that follow the triplet motor pattern

Only cycles with valid peaks for both working and balancing side superficial masseter and posterior temporalis muscles were analyzed. Channels were ordered by peak time (earliest to latest). If the first two channels were balancing side superficial masseter and working side posterior temporalis (in any order), the cycle followed the triplet motor pattern. This process was repeated for onset, and peak and for all valid cycles.

Based on random probability, 1/6 of all cycles should follow the triplet motor pattern. The probability that the first muscle will fit the triplet motor pattern is two out of four possible muscles. The probability that the second muscle will fit the triplet motor pattern is one out of three possible muscles. The product of these two fractions yields the probability that the cycle will follow the triplet motor pattern. One tailed binomial probability tests were used to test whether the actual probabilities were significantly ($p \leq 0.05$) different from 16.66%.

Here, the percent of cycles that follow the triplet motor pattern determines whether or not a particular species follows the triplet motor pattern more frequently than expected by random probability. Using the percent of cycles that follow the triplet motor pattern as opposed to the mean ensures that the variability in the data is appropriately captured. Comparing to random probability implies that if the triplet motor pattern occurs more frequently than expected at random then there must be a functional and/or physiological reason for it. For this portion of the study, if the percentage of cycles that follow the triplet motor pattern exceeds the percentage predicted by random probability at onset, peak, and offset, then that species follows the triplet motor pattern.

Additionally, for each cycle, the order in which muscles reach onset, peak, and offset was calculated. There are 24 total possible permutations of the four muscles. Based on random probability, each permutation will be observed 4.17% of the time. One tailed binomial probability tests were used to test whether the actual probabilities were significantly ($p \leq 0.05$) different from 4.17%. Once again, by comparing to random probability we assume that if that particular permutation occurs more frequently than expected at random, then there must be a functional and/or physiological reason for it.

RESULTS

Mean onset, peak, and offset time

Table 2.4. Mean onset, peak, and offset time relative to wSM (ms)

		bSM	wPT	bPT
Eulemur	Onset	5.35 ± 34.48*	5.53 ± 12.53*	-8.08 ± 18.80*
	Peak	2.91 ±12.37*	2.43 ± 10.81*	-9.87 ± 13.88*
	Offset	-1.36 ± 22.24	-2.11 ± 15.33*	13.57 ± 23.02*
Propithecus	Onset	15.50 ± 21.51*	-15.68 ± 21.88*	-28.83 ± 46.07*
	Peak	15.66 ± 14.78*	15.12 ± 10.14*	-17.19 ± 13.00*
	Offset	7.00 ± 22.59*	37.56 ± 14.79*	6.91 ± 12.17*
Papio	Onset	26.46 ± 39.31*	-10.33 ± 17.41*	-51.31 ± 15.40*
	Peak	16.23 ± 19.18*	6.32 ± 15.28*	-28.09 ± 14.30*
	Offset	12.53 ± 19.85*	19.68 ± 23.29*	-13.08 ± 29.02*
Macaca	Onset	5.76 ± 17.27*	-0.11 ± 15.87	-34.76 ± 15.63*
	Peak	7.67 ± 14.47*	4.83 ± 15.06*	-20.36 ± 12.17*
	Offset	7.10 ± 12.75*	7.26 ± 14.60*	-9.57 ± 10.54*
Pan	Onset	-13.13 ± 39.35*	22.34 ± 35.66*	9.17 ± 42.27*
	Peak	5.31 ± 35.40*	13.73 ± 36.82	0.71 ± 39.63
	Offset	-2.95 ± 24.80	-7.35 ± 25.99*	-7.26 ± 33.19*

Table 2.4 The mean onset, peak, and offset time relative to wSM in milliseconds. A t-test was used to determine whether the values were significantly different from 0 ms.

Table 4 shows the mean onset, peak, and offset times for the jaw elevators relative to the working side superficial masseter (wSM) for all species. Using these mean values of onset, peak and offset, *Eulemur* follows the triplet motor pattern at onset and peak but

not offset. Although the timing of bSM activity is more variable than that of wPT, the mean onset, peak, and offset times for both muscles are separated by 0.75 ms or less. In *Eulemur*, bPT is consistently the last muscle to reach onset, peak, and offset.

Propithecus follow the triplet motor pattern at peak and offset but not onset. Based on the mean onset time, wPT reaches onset before wSM. However, the muscles reach peak activity in the following order: bSM, wPT, wSM, bPT. Although the triplet motor pattern is maintained during offset, wSM reaches offset after bPT. The mean offset times of bPT and bSM are separated by just 0.09 ms.

Papio follow the triplet motor pattern at peak and offset but not onset. Based on the mean onset, peak, and offset times for *Papio*, bSM is the first muscle to fire, followed by wSM. The two PTs fire last, with wPT preceding bPT. The muscles reach peak in the following order: bSM, wPT, wSM, bPT. However, wPT reaches offset before bSM. All onset peak and offset times were significantly different from 0 ms in *Papio*.

Like *Propithecus* and *Papio*, *Macaca* also follow the triplet motor pattern at peak and offset but not at onset. The mean onset time for wPT is just -0.11 ± 15.87 ms after wSM. The distribution of wPT onset times in *Macaca* is not significantly different from 0 ms. The muscles reach peak in the following order: bSM, wPT, wSM, bPT. Once again, like *Propithecus* and *Papio*, bPT reaches offset before wSM.

Pan follow the triplet motor pattern at peak activity only. The mean onset times for wPT and bPT are earlier than the onset time of wSM and bSM. The onset times for bSM, wPT, and bPT are all significantly different from 0 ms. In fact, bSM is the last muscle to reach onset. The muscles reach peak in the following order: wPT, bSM, bPT, wSM. However, the distribution of peak activity time for bPT is not significantly different from wSM. Thus the two muscles in triplet II may reach peak close to simultaneously. The muscles reach offset in the following order: wSM, bSM, bPT, wPT. During offset, the time distribution for bSM is not significantly different from 0 ms.

What percentage of cycles follows the triplet motor pattern?

The proportion of cycles that follow the triplet pattern at onset, peak, and offset is illustrated in Figure 3. A cycle follows the triplet motor pattern if all the channels in triplet I (bSM, wPT) reach peak, onset, *or* offset before all the muscles in triplet II (wSM, bBT); i.e., for each cycle, the triplet motor pattern was assessed separately at onset, peak and offset.

In *Eulemur*, 29% of all cycles followed the triplet motor pattern at onset, while 24% of cycles followed the triplet motor pattern at peak and 21% of cycles followed the triplet pattern at offset. The proportions were significantly different from random at onset and peak but not at offset ($p < 0.01$, $p = 0.01$, and $p = 0.05$ respectively). 17.92 % of cycles followed the triplet motor pattern both at onset and peak while 12.92 % of cycles followed the triplet motor pattern at both peak and offset. Only 8.33 % of cycles followed the triplet motor pattern at onset, peak, and offset.

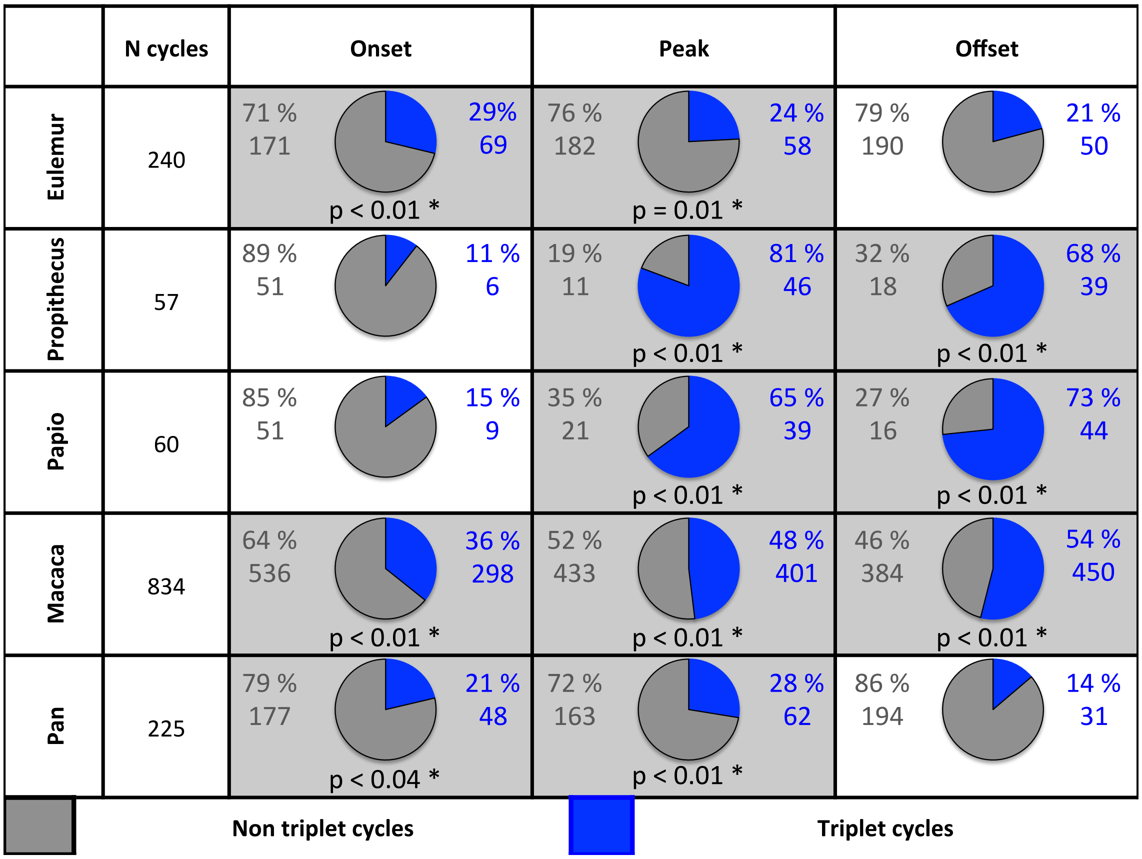


Figure 2.4 The number of cycles per species that follow the triplet motor pattern. The column labeled "N cycles" shows the total number of cycles per species. Each species has three pie charts, one each for onset, peak, and offset. Cycles that follow the triplet motor pattern are shown in blue while cycles that do not follow the triplet motor pattern are shown in gray. The number and percentage to the left of each pie chart represent the number and percent of cycles that do not follow the triplet motor pattern. The number and percentage to the right of each pie chart represent the number and percentage of cycles that do follow the triplet motor pattern. If the number of cycles that follow the triplet motor pattern are significantly greater ($p \leq 0.05$) than the number expected by random probability (16.66%) then the p-value is depicted below the pie chart and the box is shaded.

The second strepsirrhine species studied, *Propithecus,* had the smallest sample size with 57 cycles. In *Propithecus*, 11% of cycles followed the triplet motor pattern at onset, 81% of cycles followed the triplet motor pattern at peak amplitude and 68% followed the triplet motor pattern at offset. Both peak and offset were significantly different from random probability ($p < 0.01$ for both). Only 7.02% % of cycles followed the triplet motor pattern at onset, peak, and offset. 10.53 % of the cycles followed the triplet motor pattern both at onset and peak while 57.89 % of cycles followed the triplet motor pattern at both peak and offset. Hence, *Propithecus* followed the triplet motor pattern at peak muscle activation and at offset.

Among the anthropoids, *Macaca* followed the triplet motor pattern at onset (36% of cycles), peak (48%), and offset (54%) (p values < 0.01). 29.02 % of cycles followed the triplet motor pattern both at onset and peak while 37.53 % of cycles followed the triplet motor pattern at both peak and offset. Only 22.06 % of cycles followed the triplet motor pattern at onset, peak, and offset. *Papio* followed the triplet motor pattern at peak (65 %, $p < 0.01$) and offset (73 % $p < 0.01$) but not at onset (15 %). All 15% of cycles that followed the triplet motor pattern at onset, also followed it at peak, while 46.67% of cycles that followed the triplet motor pattern at peak also followed the triplet motor pattern at offset. However, only 5% of cycles followed the triplet motor pattern at onset, peak, and offset. These findings suggest that cycles are more likely to follow the triplet motor pattern at one or two points in the cycle than to maintain the triplet motor pattern throughout the cycle.

Pan, the only hominid studied followed the triplet motor pattern at onset and peak but not offset. 21 % of cycles followed the triplet motor pattern at offset, 28% followed the motor pattern at peak, and 14 % followed the triplet motor pattern at offset. In fact at offset, the number of cycles that followed the triplet motor pattern was significantly less than that expected by random probability. Only 8 % of cycles followed the triplet motor pattern at onset and peak, while only 5 % of cycles followed the triplet motor pattern at peak and offset. Only 2.22% of cycles followed the triplet motor pattern at onset, peak, and offset.

Specific motor patterns occurred more frequently than expected by random probability in all five species. In both *Eulemur* and *Macaca,* the same four permutations occurred more often than expected by random probability at onset, peak, and offset. These four permutations were wPT, wSM, bSM, bPT; wPT, bSM, wSM, bPT; bSM, wSM, wPT, bPT; bSM, wPT, wSM, bPT. Two of these motor patterns follow the triplet motor pattern. Two permutations begin with wPT and end in bPT, one follows the triplet motor pattern but in the second, wSM fires before bSM. However, the temporal offset between the two muscles is not known. It is possible that the two SMs fire simultaneously. In the final sequence that occurs more frequently than expected by random probability at all three time points, the two SMs reach onset, peak, and offset before the two PT. Additionally, in *Eulemur,* the permutation wPT, wSM, bPT, bSM occurs more frequently than expected by random probability at onset and offset but not at peak. In Macaca, the permutation wSM, bSM, bPT, wPT occurs more frequently than expected by random probability at onset and peak but not offset.

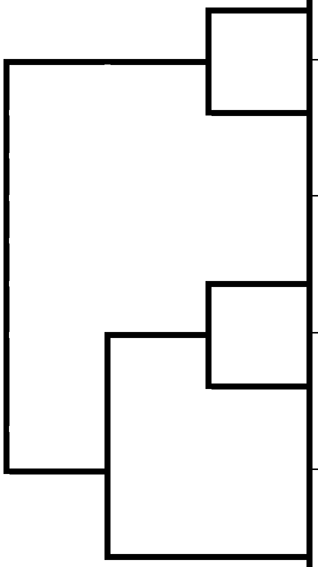
In *Propithecus*, no cycles are observed more frequently than expected by random probability at all three time points. However, two triplet permutations do occur more frequently than expected by random probability at two out of three time points. In *Papio,* just one triplet permutation occurs more frequently than expected by random probability at all three time points. One more triplet permutation occurs more frequently than expected by random probability at peak and offset but not at onset. Finally, the permutation bSM, wSM, wPT, bPT occurs more frequently than expected by random probability at onset and peak but not at offset. Pan was unlike the other species because triplet permutations did not occur more frequently than expected by random probability at any of the time points. Instead the permutation wSM, bSM, bPT, wPT occurs more frequently than expected by random probability at onset, peak, and offset. Three additional permutations occur more frequently than expected by random probability at onset and peak but not offset. All three of these permutations start with wPT. In one sequence the second muscle is wSM while in the other two it is bPT. Finally, the permutation wSM, wPT, bSM, bPT occurs more frequently than expected by random probability at peak and offset in *Pan* but not at onset.

Table 2.5 Order in which muscles reached onset, peak, and offset. Muscle 1 is bSM, 2 is wPT, 3 is wSM, 4 is bPT. The number of cycles that fit each permutation at onset, peak, and offset are listed. Shaded boxes and values followed by asterisk indicate that the triplet motor pattern was observed more frequently than expected by random probability based on one-tailed binomial probability. Out of 24 possible permutations, the random probability of observing a specific motor pattern is 4.17%

Table 2.5 Permutations in muscle order at onset, peak, and offset

	Eulemur			Propithecus			Papio			Macaca			Pan		
	On	Pk	Off	On	Pk	Off	On	Pk	Off	On	Pk	Off	On	Pk	Off
'4321'	1		5					1				1	7		6
'4312'	1	2	1	2							1		2	2	12
'4231'	3	1	3						2				33*	3	4
'4213'	6	3										1	12	10	5
'4123'	3	7	8	4									6	7	5
'4132'		2	3	3								2	4	7	14
'3421'	3		2								2	5	3	7	6
'3412'		2	2					1	1			3	1	2	4
'3241'	11	3	13							1	1	5	7	4	10
'3214'	11	11	16*		3		1	2		68*	44	43	7	16*	22*
'3124'	11	5	9	7*			7*	2		111*	71*	44	1	7	13
'3142'	3	1	6						1	1	5	9	3	2	19*
'2341'	19*	15	20*			3			3	9	9	27	30*	17*	5
'2314'	48*	47*	28*		5	3	2		5	141*	114*	78*	23*	24*	16*
'2431'	9	8	3			12*		1	1		4	8	26*	16*	6
'2413'	12	5	2			9*			1		3	30	18*	19*	9
'2143'	15	12	11		2	16*			4		18	48*	10	16*	7
'2134'	18*	26*	18*		22*	9*	1	8*	16*	84*	153*	131*	14	7	7
'1324'	21*	46*	43*	25*	3		41*	14*	1	197*	160*	97*	2	9	10
'1342'	5	6	7	5					1	5	11	36	1	9	16*
'1234'	26*	16*	17*	6*	19*	5	7*	31*	22*	217*	224*	216*	4	12	6
'1243'	5	13	10		3		1		2		7	29	6	10	4
'1423'	6	7	10	1							4	5	2	9	8
'1432'	3	2	3	4							3	16	3	10	11
TOTAL	240			57			60			834			225		

Table 2.6



	Mean time			Percent triplets		
	Onset	Peak	Offset	Onset	Peak	Offset
Eulemur	▨	▨		▨	▨	
Propithecus		▨	▨		▨	▨
Papio		▨	▨		▨	▨
Macaca		▨	▨	▨	▨	▨
Pan		▨		▨	▨	

Table 2.6 Shaded boxes represent a corroborated hypothesis where the triplet motor pattern was observed.

DISCUSSION

The purpose of this study was to quantify variability in jaw elevator motor patterns using two different metrics to determine which species follow the triplet motor pattern at three different times in the masticatory cycle: onset of muscle activity, peak muscle activity, and offset of muscle activity. The species studied included *Propithecus verreauxi*, *Papio anubis*, *Macaca fuscata*, *Eulemur fulvus*, and *Pan troglodytes.* Following previous workers, the mean onset, peak, and offset times were calculated relative to wSM (Hylander et al. 2005; Vinyard et al. 2005). This technique generates a representative statistic (the mean) for all cycles in the species, resulting in a binary observation on the presence/absence of triplets. It does not take into consideration both the inter-cycle and intra-cycle variance in the triplet motor pattern. The second method estimates from the available data, the proportion of cycles per species following the triplet motor pattern. This second method asks whether the number of triplet cycles differs from that predicted at random. This method accounts for both inter-individual variation and intra-individual variation. It accounts for the fact that (1) multiple motor patterns produce the force and kinematics required to mechanically digest food during mastication; and (2) the motor pattern is continuously modified based on sensorimotor feedback, even within a single masticatory cycle. Therefore, the motor pattern changes not only within species, but also within individuals, and even within individual cycles. The results of this study are summarized in Table 5.

Two methods were used to evaluate whether the triplet motor pattern was present in each of the five species. Both methods yield similar results for *Eulemur, Propithecus* and *Papio*. For *Macaca,* more cycles than expected by random probability follow the triplet motor pattern at onset. Out of a total of 834 macaque cycles analyzed, wSM (triplet II) reached onset before wPT (triplet I) in just 41 cycles. Based solely on the mean, *Macaca* do not follow the triplet motor pattern at onset, although 36% of all jaw elevator cycles comply with the triplet motor pattern at onset. Similarly, in *Pan*, significantly more cycles follow the triplet motor pattern at onset than expected by random probability. However, because 21% of cycles display this motor pattern, the trend is not captured by the mean. Hence, the mean onset, peak, and offset times do not capture the variability in muscle onset, peak, and offset times.

All five primate species studied exhibited the triplet motor pattern at peak activation in a significant proportion of cycles. These findings corroborate Weijs's (1994) triplet hypothesis at peak muscle activation only. These findings suggest that there may be a functional reason for maintenance of the triplet motor pattern at peak muscle activation in primates. Among the anthropoids, the two Old World monkeys analyzed, *Papio* and *Macaca*, also exhibited the triplet motor pattern at peak and offset. Moreover, presentation of the triplet motor pattern at peak and offset appears to be a convergent character shared by non-hominid anthropoids. *Propithecus,* a strepsirrhine species that independently evolved a fused mandibular symphysis, also follows the triplet motor pattern at peak and offset but not at onset. *Eulemur*, the only species studied with an unfused symphysis was the only species whose mean onset time followed the triplet motor pattern. Further studies including a larger range of species are needed to confirm these findings; however, we hypothesize that the presence of the triplet motor pattern at peak and offset is a convergent trait for non-hominid primates with a fused symphysis.

Pan, the only hominid species studied, exhibits the triplet motor pattern only at mean peak time and is the only anthropoid species studied that does not have more triplet cycles at offset than expected at random probability. While chimps have never been previously tested for the triplet motor pattern, their close relative, *Homo*, does follow the triplet motor pattern at peak muscle activation (Moller 1966; Langenbach and Hannam 1999; Williams 2011). Incidentally, *Pan* is both the only hominid species included in the present study and the only species with a fused mandibular symphysis that does not demonstrate the triplet motor pattern at offset.

These findings demonstrate that the standard practice of calculating the mean onset, peak, and offset time does not capture the variation in muscle activation patterns used by primates at different times in the cycle and in different cycles. What is the significance of this variation? One possibility is that the triplet motor pattern acts as an “attractor” to the masticatory system; i.e., a state or behavior toward which a system progresses regardless of the initial state (American Heritage Dictionary of the English Language, 2000). This hypothesis suggests that if all external forces and factors acting on the system were constant, the triplet motor pattern would be one among a discrete and finite set of motor pattern observed. However, modifications to the motor pattern may be necessary to maintain kinematics given changing conditions, such as food bolus, condition and position. The above hypothesis is bolstered by the fact that in four out of the species, the permutations bSM, wPT, wSM, bPT and wPT, bSM, wSM, bPT occur more frequently than expected by random probability at at least two time points during the chewing cycle. These permutations were followed in frequency by wPT, wSM, bSM, bPT which was observed more frequently than expected at onset, peak, and offset in three out of five species and the permutation bSM, wSM, wPT, bPT which occurred more frequently than expected by random probability during at least two time points in three out of five species.

As a result, the triplet motor pattern may be one of multiple motor patterns that produce a given pattern of kinematics and force production, especially during offset and onset. Currently, it is unclear what conditions contribute to the triplet motor pattern or its alternatives being generated/selected by the central nervous system. These conditions may include variations in kinematics related to food type and temporal position in the masticatory sequence (early vs. late) (Reed and Ross, 2010). Nonetheless, in all four of the most frequently observed motor patterns, wPT fires before bPT. PT is thought to have less of a wishboning affect compared to SM and MPt and is thought to produce a predominantly vertical force (Hylander and Johnson 1994). Hence, cycles in which wPT fires before bPT but the wSM fires before bPT, or cycles where both PT fire before both SM would be hypothesized to have decreased wishboning and transverse rotation and may be utilized when increased transverse forces are not needed.

Alternately, there might not be obvious or predominant relationships between food type-related variance in jaw kinematics and muscle motor patterns because of redundancy in the masticatory system—multiple patterns of muscle activation can generate similar patterns of force production and kinematics (Van Eijden et al. 1990; Vinyard et al., 2008). This hypothesis is supported in species where the mean onset, peak, or offset times do not follow the triplet motor pattern but the proportion of cycles that follow the triplet motor pattern is higher than expected by random probability. One or two jaw elevator muscles reaching onset, peak, or offset milliseconds too early or too late modify the motor pattern but may not appreciably affect the kinematics. In this case, motor patterns could only be considered to be conserved if the variation in kinematics is disproportionately larger than the variation in motor patterns: to address this possibility, future work should examine variation in muscle activity patterns relative to variation in jaw kinematics. Previous workers have suggested, based on qualitative observations, that jaw kinematics do vary more than triplet motor units (Hiiemae 1978), but this has yet to be proven quantitatively. If the variation in kinematics is less than or proportional to the variation in kinematics, then it is possible that the central nervous system is not wed to specific motor patterns but is more concerned with modulating force production and kinematics to achieve specific goals. If the variation in jaw kinematics is equal to the variation in motor patterns, the goal itself may be changing during the masticatory sequence. Directed changes in force production and kinematics may be correlated with changing bolus properties and points of tooth contact.

It has been suggested that mammals display broad similarities in motor patterns during mastication (Hiiemae 1978; Bramble and Wake 1985; Weijs 1994). Asymmetrical activity of the bilateral jaw elevator muscles (Herring 1973 1976 1979; Gorniak 1977 1985; Weijs and Dantuma 1980) was reified into a triplet motor pattern, ancestral for mammals, and modified by natural selection (Weijs, 1994). Many workers in the field have used the mean peak activation time of triplet muscles to test for the presence of triplets in a variety of mammals. The current study found that the *mean* peak activity times for the jaw elevator muscles follow the triplet motor pattern in all five species studied. The *prevalence* of the triplet motor pattern is also greatest at peak muscle activation compared to onset and offset. However, there was variability in the prevalence of the triplet motor pattern between species (ranging from 20% in *Pan* to 81% in *Propithecus* at peak muscle activation). Flexibility in muscle activation patterns indicates that the jaw elevator motor pattern is not a fixed binomial trait. There is variability between cycles and the motor pattern can be modified between onset, peak and offset. Further studies are needed to understand the implications of this variability for motor control by the CNS.

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DATA AVAILABILITY

Wall, C. E., Vinyard, C. J., Williams, S. H., Gapeyev, V., Liu, X., Lapp, H., and German, R. Z. 2011. Overview of FEED, the Feeding Experiments End-User Database. Integrative and Comparative Biology 51(2): 215-223. http://dx.doi.org/10.1093/icb/icr047.

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JAW ELEVATOR MUSCLE COORDINATION DURING RHYTHMIC MASTICATION IN PRIMATES: ARE TRIPLETS UNITS OF MOTOR CONTROL?

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ABSTRACT

The activity of mammal jaw elevator muscles is often described using the concept of the triplet motor pattern. Weijs (1994) hypothesized that triplet I is consistently activated before triplet II and each triplet of muscles is recruited and modulated as a unit. In this study new, mathematically precise definitions of unison, synchrony, and coordination are used to test the following hypotheses: muscles in the same triplet are active more in unison than muscles in two different triplets; activity of muscles in the same triplet is more synchronized than activity of muscles in two different triplets; and muscles in the same triplet are more tightly coordinated than muscles in two different triplets. Data from *Propithecus verreauxi*, *Papio anubis*, *Macaca fuscata, Eulemur fulvus*, and *Pan troglodytes* were analyzed using measures of continuous and relative phase. Triplet I but not triplet II muscles were more in unison than non-triplet muscles in *Eulemur, Papio,* and *Macaca*. Triplet I muscles were more synchronized than non-triplet muscles only in *Macaca*, and triplet II was more synchronized than non-triplet muscles only in *Pan*. Triplet muscles were more coordinated during triplet cycles than non-triplet cycles, while non-triplet muscle pairs were more coordinated during non-triplet cycles than triplet cycles. These results suggest that, the CNS may recruit the triplet muscles as a unit during triplet cycles. However, a different pattern of muscle coordination exists during non-triplet cycles. These results are congruent with the notion that stereotyped motor patterns, like triplet motor patterns, provide a way for the CNS to bootstrap complex problems of optimal control during rhythmic mastication based on sensory feedback.

INTRODUCTION

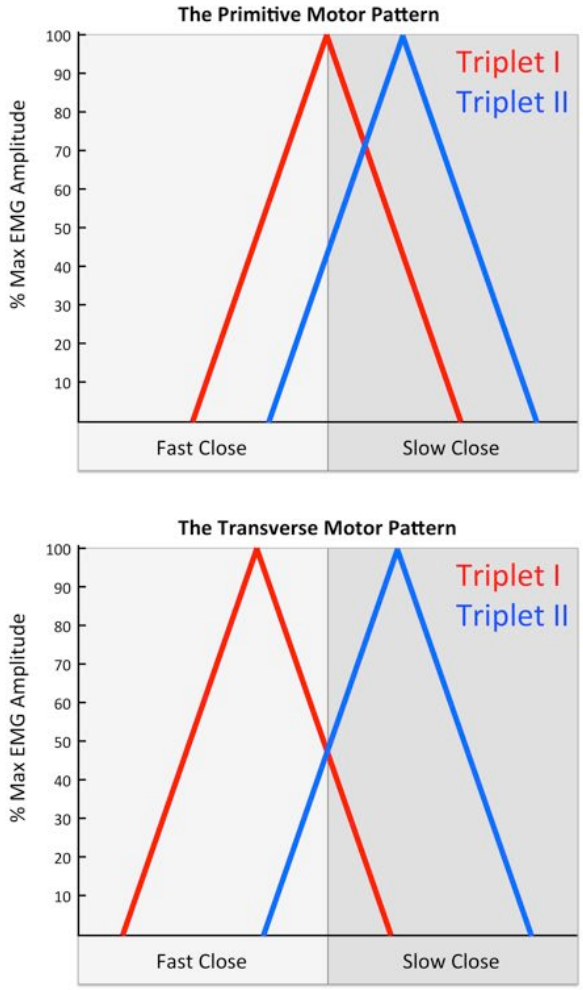


Figure 3.1. The triplet hypothesis Weijs (1994) hypothesized that jaw elevation in primates is produced by a combination of movement modules known as triplet I and triplet II. He predicted that the primitive mammalian motor pattern (A) is observable in extant strepsirrhines while extant anthropoids display the transverse motor pattern (B).

Mastication is the cyclic chewing behavior distinctive of mammals. Many species of animals chew, including lizards, birds, and fish (e.g. Gidmark et al. 2014), but the hallmarks of mastication primitively and in many extant mammals include: precise occlusion between the teeth; transverse tooth and jaw movements during food breakdown; and unilateral application of force to the food item on the working side (ws) of the toothrow (the non-biting side is the balancing side (bs) (Hiiemae 1976; Williams et al. 2011). The transverse movements during jaw elevation are produced by asymmetric activation (amplitude and timing) of the jaw elevator muscles. In mammalian mastication the three muscles hypothesized to be primarily responsible for the lateral-to-medial ws jaw movements during jaw elevation are the superficial masseters (SM), posterior temporales (PT), and medial pterygoids (MP). Building on previous research (Herring 1973 1976 1979; Gorniak 1977 1985; Weijs and Dantuma 1980), Weijs (1994) hypothesized that the bilateral activity of these three muscles is captured by the concept of a *triplet motor pattern* (Figure 1) in which the bSM, bMP, and wPT are activated first and form triplet I, while the wSM, wMP, and bPT are activated second and form triplet II. Primitively, the activity of these two triplets is hypothesized to follow the activity of symmetrical vertical closers (SVCs) at the start of jaw elevation. Following activity of the SVCs, triplet I is activated slightly before triplet II during jaw elevation, rotating the jaw towards the working side, then triplet II stays active later than triplet I, thereby rotating the jaw towards the balancing side, producing the transverse jaw movements characteristic of mammals.

Weijs suggested that each species of mammal has a limited set of muscle activation patterns represented in the brainstem, but that variability in the motor pattern can be produced by sensory feedback (Weijs 1994). Indeed, analyses of variance in muscle EMG activity patterns and jaw kinematics show that there is more variation within chewing sequences on a single piece of food than between chewing sequences on different foods (Vinyard et al. 2008; Ross et al. 2012; Ross and Iriarte-Diaz 2014). This variability raises important questions about the nature of the variability in jaw elevator motor patterns during mastication and its implications for motor control by the CNS. The goal of the present study is to ask: do jaw elevator muscle triplets function as units of motor control? This question is addressed by evaluating the degree to which the CNS co-modulates the activity of jaw elevator muscle pairs in triplets more closely than it does non-triplet pairs. Three specific hypotheses were tested:

1. Muscles in the same triplet are active more in unison than muscles in two different triplets;

2. Activity of muscles in the same triplet is more tightly synchronized than activity of muscles in two different triplets;
3. Muscles in the same triplet are more closely coordinated than muscles in two different triplets.

Definitions and mathematical tools

Testing hypotheses about cyclic phenomena benefits from use of mathematical tools used to study oscillations in dynamical systems: specifically, *continuous phase* (ϕ), a continuous and cumulative measure of an oscillator's progress through its cycle, and *relative phase* ($\phi_{1\text{-}2}$), the relative timing of two oscillators. A 0° $\phi_{1\text{-}2}$ indicates that two signals are perfectly *in-phase synchronized* (or in *unison* with one another) while a 180° $\phi_{1\text{-}2}$ indicates that two signals are perfectly anti-phase synchronized.

Relative phase ($\phi_{1\text{-}2}$) is used in both dynamical systems and neuroscience to quantify synchrony (Pikovsky et al. 2001; Le Van Quyen et al. 2001). In the study of dynamical systems, synchronization is a *process*, the "adjustment of rhythms due to an interaction" (Pikovsky 2001, xviii), characterized by frequency entrainment and phase locking (Pikovsky 2001). In the current study, two muscles are defined as being in synchrony if specific events in their cycles (e.g. activity onset, peak, and offset) repeatedly occur with the same $\phi_{1\text{-}2}$. Activity in two muscles is synchronized when specific events in their activity cycle exhibit a constant $\phi_{1\text{-}2}$. Synchrony includes the case when two muscles reach specific events in their cycles—e.g., onset, peak, and offset—at the *same phase* in their cycles ($\phi_{1\text{-}2} = 0$). We refer to this subset of synchrony as *unison* and measure it by comparing the mean $\phi_{1\text{-}2}$ of two muscle pairs to the predicted $\phi_{1\text{-}2}$ of 0°.

Synchrony also includes the more common cases when *different events* in the activity cycles of two muscles consistently occur at the *same phase*. For example, during locomotion, maximum *flexion* of the left shoulder is synchronized with maximum *extension* of the right shoulder. It is important to note that synchrony is not all or nothing: statistical analyses can reveal relative degrees of synchrony. In the present case, activity in two muscles is perfectly synchronized if there is no variance in $\phi_{1\text{-}2}$ across all events (i.e. $\phi_{1\text{-}2}$ is constant but not necessarily equal to 0°), but of course, two muscles are never perfectly synchronized and the magnitude of variance in $\phi_{1\text{-}2}$ is a measure of the degree to which two oscillators are synchronized. Hence there are degrees or strengths of synchronization. This definition of a state of synchrony is compatible with the process of synchronization described by Pikovsky et al. (2001) because strong synchrony implies (a) mechanism(s) of synchronization. At present we are agnostic as to these mechanisms and only ask whether there is evidence of such synchronization in jaw elevator muscle triplets.

Coordination is a term often employed to describe interactions between multiple elements in a biological system, however strict definitions and standards for quantifying coordination are seldom applied. For example, Wainwright et al. (2008) define coordination rather loosely as "association between movements of different body parts" (Wainwright 2008, 3524). Here we argue for a more precise and quantifiable definition of coordination that emphasizes the implication that coordination involves *active modulation of relative timing and amplitude*. We define muscle coordination as the *modulation (adjustment or maintenance)* of relative muscle activity (*amplitude and/or timing*) to achieve goal specific force production and kinematics (Konczak et al. 1997). Even though one may not know what goals are actually being met, we argue that active modulation—coordination—is implied if the system co-modulates the activity of jaw elevator muscles in the context of variation in muscle firing patterns. In the present case, variation in muscle firing patterns within chewing sequences facilitates application of force and displacement to the food in such a way that it is broken down into a swallow-safe bolus while minimizing tooth wear and the risk of tooth breakage. Hence, we propose that coordination between two muscles be quantified as the correlation coefficient, $\rho(\phi_1, \phi_2)$ between the continuous phase (ϕ) of two muscles. Therefore, $\rho(\phi_1, \phi_2)$ measures two muscles' tendency toward co-modulation despite naturally occurring (unquantified) perturbations. Perfect unison and perfect synchrony can be subsets and manifestations of coordination. However it is possible for two muscles to be coordinated without being synchronized or in unison if they both accumulate phase at different rates without modulating their activity with each other.

With these definitions in mind, this paper tests the following hypotheses regarding muscle activity patterns in jaw elevator muscle triplets.

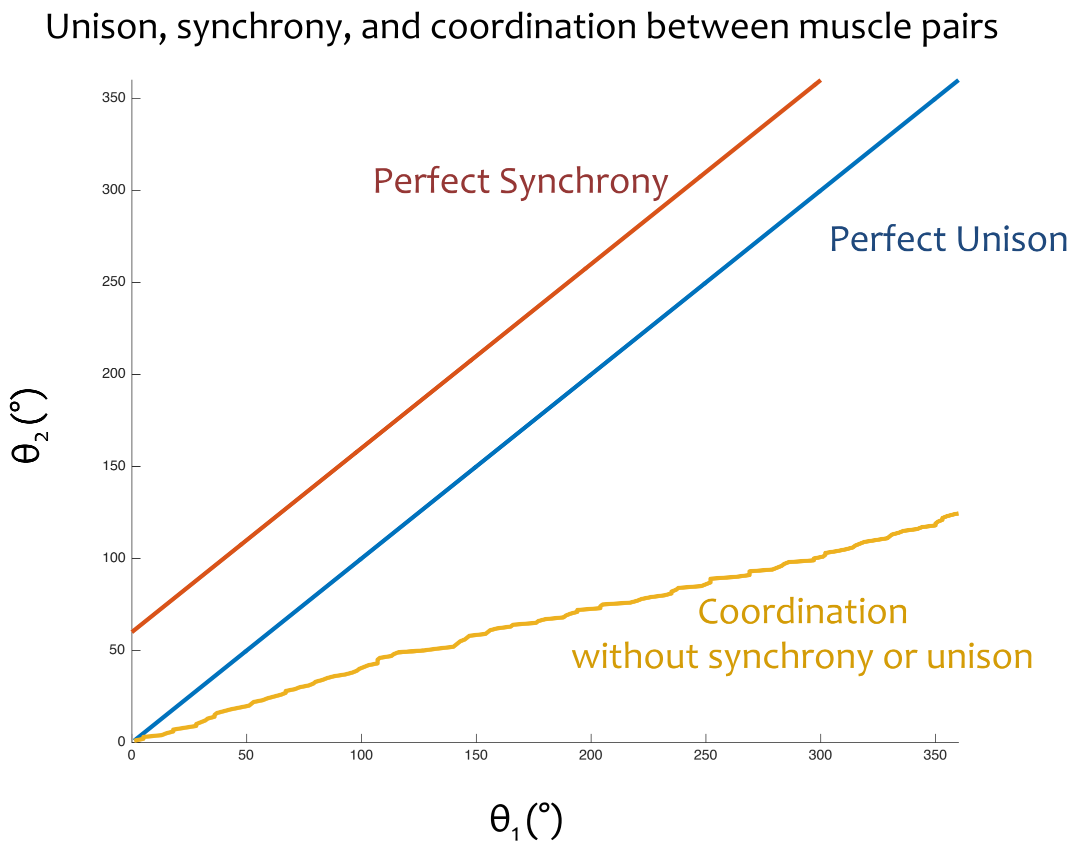


Figure 3.2 Continuous phase of two muscles that are in unison, synchrony, and coordinated are plotted in the figure above. The blue line shows the relative phase of two muscles that are perfectly in unison with each other. The red line shows the relative phase of two muscles that are perfectly synchronized with each other but not in unison. Finally, the yellow line shows the relationship between two muscles that are coordinated but not in unison or synchrony. Perfect unison and synchrony can be subsets of coordination.

Activity of muscles in the same triplet is more tightly synchronized than activity of muscles in two different triplets. This hypothesis predicts that muscles in the same triplet have a less variable $\phi_{1\text{-}2}$ than muscles in two different triplets, where variation in $\phi_{1\text{-}2}$ is measured as $\bar{\sigma}_{\phi_{1-2}}$. This hypothesis addresses the possibility that although triplets may not reach onset, peak, and offset at the same time—in unison (see previous hypothesis)—they may be synchronized in maintaining constant *relative timing*.

Muscles in the same triplet are more closely coordinated than muscles in two different triplets: This hypothesis predicts that muscles in the same triplet will co-modulate their activity—have a higher $\rho(\phi_1, \phi_2)$—than muscles in two different triplets. This hypothesis addresses the possibility that, although triplet muscles may not be active in unison, or be synchronized, their activity may still be co-modulated by the CNS.

Falsification of these hypotheses would argue against the idea that triplets I and II are units of control for the CNS.

MATERIALS AND METHODS

Data selection

Data used in this study from *Propithecus verreauxi, Papio anubis*, and *Macaca fuscata* were downloaded from the FEED database (Wall et al. 2011). Data for *Eulemur fulvus* and *Pan troglodytes* were extracted from data files previously collected by one of us (CFR) in connection with other studies. Sequences were selected for analysis if chewing side could be identified and the EMG data included the four triplet muscles analyzed and were of good quality (not clipped or too noisy). Sequences from the FEED database that had EMG values for bSM, wSM, bPT, and wPT were utilized in this study. If chewing sequences in the FEED database included two channels of recordings from the same muscle, one of the two signals was arbitrarily chosen based on the following criteria: most constant baseline, least baseline noise, and largest unclipped amplitude during rhythmic mastication.

Table 3.1. Number of Cycles per Individual

Species	Individual	N cycles with triplets	N cycles total
Eulemur fulvus	B	17	60
	H	10	71
Propithecus verreauxi	C	46	57
Papio anubis	M	10	14
	G	5	6
	C	8	15
Macaca fuscata	B	76	172
	S	35	119
	T	101	183
Pan troglodytes	O	13	43
	A	21	84

Table 3.1 Number of cycles per individual

Chewing sequences from *Macaca, Papio*, and *Propithecus* were marked in the FEED database as exclusively left or right chews. For *Eulemur* chewing side was recorded on the voice track of the tape recorder or in experimental notes during data collection and corroborated using changes in principal strain orientation recorded from the mandible. For *Pan*, the chew side was determined from the direction of jaw movement during the slow close phase of the gape cycle as seen on videos of the recording session: if the mandible was moving towards the left, then it was a right chew and vice-versa. However, jaw movement was not visible for all cycles, so for the remaining cycles, a clustering algorithm that utilized EMG data for all jaw elevator muscles was used to determine the ws. The clustering algorithm successfully classified all those cycles for which ws could be seen on video, so we proceeded under the assumption that all cycles in the two clusters were correctly assigned to ws.

Pre-processing

The data were full wave rectified and a 4th order low pass Butterworth filter with cutoff at 30 Hz was applied, followed by a root mean square (RMS) moving window integration with a 42 ms rectangular window moving one point at a time (Hylander 1984). Data were collected at 10 K Hz except for *Eulemur* which was collected at 1 K Hz and which was collected at 20 K Hz. Cycle start and end was chosen manually such that all four channels had minimal EMG activity at the start of the cycle and each channel reached peak amplitude only once during each cycle. Every cycle was manually reviewed to ensure that no channels were clipped and all cycles represented only one complete cycle of jaw closing EMGs. FEED database characterization of left and right chews was used to change muscle names to include ws and bs. For Ross lab data, the experiment data sheet was used to identify the working and balancing sides and change the left and right superficial masseter and posterior temporalis to working and balancing side.

Calculation of continuous phase, ϕ

Channel amplitudes were normalized from -1 to 1 and all cycle lengths were normalized to 100 frames. The beginning and end of each sequence was padded with 1000 zeros then the Hilbert transform was used to calculate continuous phase. The Hilbert transform H(t) of a signal is the convolution of the signal u(t) with a filter h(t) = 1πt (equation 1).

Equation 1

$$H(t) = \frac{1}{\pi}\int_{-\infty}^{\infty} \frac{u(\boldsymbol{\tau})}{(t-\tau)}\, d\tau$$

The result of the Hilbert transform is an analytic signal with a real component that is equal to the original signal and an imaginary component equal to the convolution described above. For this study, the Matlab command “hilbert” was used to obtain this analytic signal. The continuous phase of the signal is equal to the arctangent of the real part of the analytic signal divided by the imaginary portion of the analytic signal (equation 2).

Equation 2

$$\phi = arctan\left(\frac{real(H(t))}{imaginary(h(t))}\right)$$

The resulting signal was then unwrapped using the unwrap function in Matlab and the extra 1000 zeros were removed from the beginning and end of the signal. The vector was then divided back into individual cycles with 100 frames each. The starting point of each cycle was set to zero by subtracting the original cycle phase at point 1 from all 100 points in the cycle. Only cycles with a final cycle continuous phase between 300 degrees and 420 degrees were used for all species except *Propithecus*. The total number of degrees accumulated per cycle were consistently less than 260° and so this condition was omitted. All *Propithecus* cycles were used, regardless of final cycle continuous phase. For individual cycles, all 6 combinations of relative phase including the two SM and MP were calculated.

Unison

Only cycles that follow the triplet motor pattern were considered for this portion of the study. First, the mean relative phase ($\bar{\phi}_{1-2}$) for all muscle pairs was calculated within each cycle from the last onset time for any muscle to the first offset time for any muscle (defined as 25% of peak). The grand mean relative phase ($\bar{\bar{\phi}}_{1-2}$) for each pair was then calculated across all cycles. The p-values were calculated using a t-test to determine whether the mean RP of triplet pairs was significantly different ($p \leq 0.05$) from $0°$. For each species, two criteria were used to determine whether muscles in the same triplet are more in unison than muscles in two different triplets: (1) triplet I and triplet II muscle pairs must have a lower $\bar{\bar{\phi}}_{1-2}$ than all other muscle pairs; and (2) the one-tailed two-way t-test must show that muscles within the same triplet have a $|\bar{\phi}_{1-2}|$ distribution that is significantly different ($p \leq 0.05$) from that of all non-triplet muscle pairs. Additionally, a one-way t-test was used to test whether muscles within the same triplet have a $\bar{\phi}_{1-2}$ distribution that is not significantly different ($p \leq 0.05$) from 0°.

Synchrony

Only cycles that follow the triplet motor pattern were considered for this portion of the study. First, the standard deviation in relative phase ($\sigma_{\bar{\phi}_{1-2}}$) for all muscle pairs was calculated within each cycle from the onset of the last muscle (25% of peak amplitude) to offset of the first muscle (25% of peak amplitude). Then, the mean standard deviation in relative phase ($\bar{\sigma}_{\bar{\phi}_{1-2}}$) was calculated across all cycles. A one-tailed two-way t-test was used to make pairwise comparisons between the $\sigma_{\bar{\phi}_{1-2}}$ of muscles in the same triplet versus the muscles in two different triplets. For each species, two criteria were used to determine whether muscles with the same triplet are more synchronized than muscles in two different triplets: (1) triplet I and triplet II muscle pairs must have lower $\bar{\sigma}_{\bar{\phi}_{1-2}}$ than all other muscle pairs; and (2) the t-test must show that muscles within the same triplet have a $\sigma_{\bar{\phi}_{1-2}}$ distribution that is significantly different ($p \leq 0.05$) from all non-triplet muscle pairs.

Coordination

The degree of coordination between two muscle pairs was measured by the correlation between ϕ of two muscles across all triplet cycles. Triplet muscles were more coordinated than non-triplet muscles if the $\rho(\phi_1, \phi_2)$ of triplet muscles was higher than that of non-triplet muscles. The $\rho(\phi_1, \phi_2)$ for each muscle pair was also calculated for each cycle. A two-way t-test was used to determine if the triplet muscles had a significantly higher ($p \leq 0.05$) distribution of $\rho(\phi_1, \phi_2)$ during triplet and non-triplet cycles compared to non-triplet muscle pairs.

RESULTS

A cycle followed the triplet motor pattern if all the muscles in triplet I reach peak muscle activity before all the muscles in triplet II. Table 3 shows the number of cycles considered per individual and the number of cycles that followed the triplet motor pattern.

Unison

Figure 2 shows the $\bar{\bar{\phi}}_{1-2}$ (in degrees) for all muscle pairs in all five species. Triplet muscles were relatively more in unison if they had a lower $\bar{\bar{\phi}}_{1-2}$ than non-triplet muscles and a $|\bar{\phi}_{1-2}|$ distribution that is significantly different ($p \leq 0.05$) from all other muscle pairs. Triplet muscles were in absolute unison if the $\bar{\bar{\phi}}_{1-2}$ was not significantly different from zero ($p \leq 0.05$).

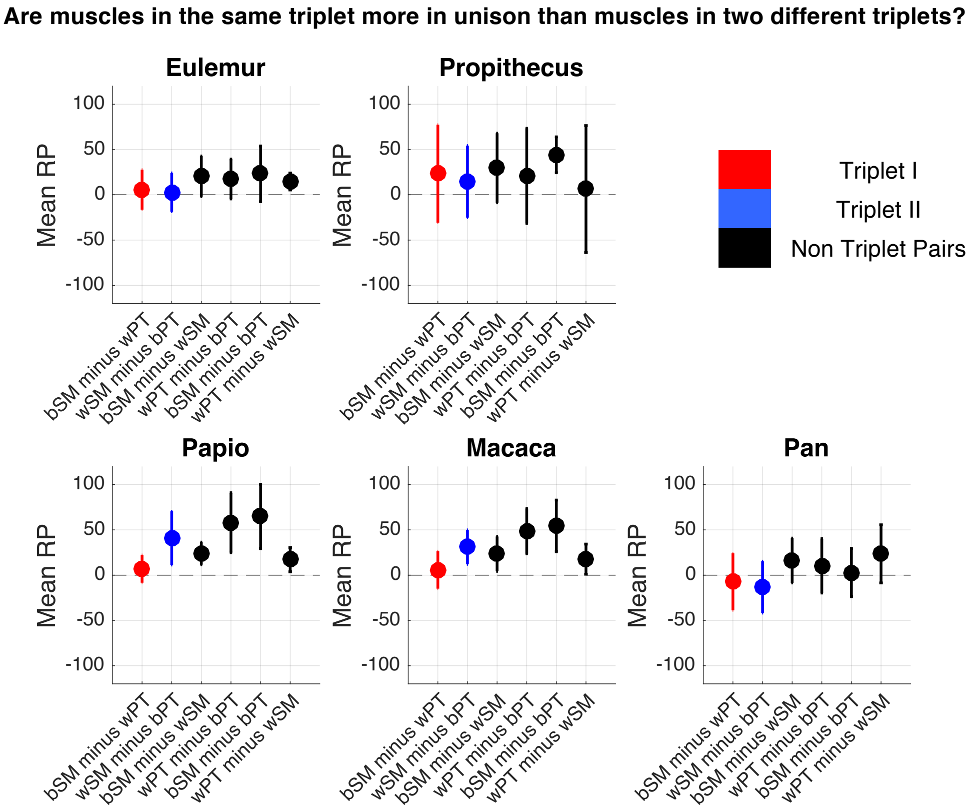
In *Eulemur,* triplet muscle pairs had the lowest $\bar{\bar{\phi}}_{1-2}$. The $|\bar{\phi}_{1-2}|$ distribution of triplet I but not triplet II was significantly different from all other muscle pairs ($p = 0.0476$ for triplet I and $p = 0.0705$ for triplet II). Both triplet pairs had a $\bar{\bar{\phi}}_{1-2}$ that was not significantly different from 0° ($p = 0.1934$ and $p = 0.5135$ for triplet I and II respectively based on a t-test). These results suggest that both triplet pairs are active in unison in *Eulemur,* but only triplet I is more in unison than non-triplet pairs.

In *Propithecus*, triplet pairs are not more in unison than non-triplet pairs. A non-triplet muscle pair (wPT - wSM) had the lowest $\bar{\bar{\phi}}_{1-2}$ (14.70°), however, this muscle pair also has the largest standard deviation in $\bar{\bar{\phi}}_{1-2}$ (70.13°). Triplet II but not triplet I has a $|\bar{\phi}_{1-2}|$ distribution that is significantly lower than all non-triplet muscle pairs ($p = 0.1248$ for triplet I and $p = 0.0062$ for triplet II). However, the $\bar{\bar{\phi}}_{1-2}$ for both muscle pairs was significantly different from 0° ($p = 0.0055$ for triplet I and $p = 0.0168$ for triplet II). Thus, for *Propithecus*, triplet muscles are not more in unison than non-triplet muscles.

In *Papio*, triplet I had the lowest $\bar{\bar{\phi}}_{1-2}$ (6.93 ± 15.30°) and a significantly lower $|\bar{\bar{\phi}}_{1-2}|$ than all non-triplet muscle pairs (triplet I $p < 0.0001$). Both triplet I and triplet II had $\bar{\phi}_{1-2}$ distributions that were significantly different from 0° ($p = 0.0407$ for triplet I and $p < 0.0001$ for triplet II). Thus in *Papio,* triplet I but not triplet II was significantly more in unison than non-triplet muscles; however, neither triplet pair was perfectly in unison.

In *Macaca*, triplet I but not triplet II has a lower $\bar{\bar{\phi}}_{1-2}$ than all other muscle pairs ($\bar{\bar{\phi}}_{1-2} = 5.78 \pm 20.70°$ for triplet I, $\bar{\bar{\phi}}_{1-2} = 30.96 \pm 19.00°$ for triplet II). Triplet I also has a lower $|\bar{\bar{\phi}}_{1-2}|$ distribution than non-triplet muscle pairs ($p < 0.0001$ for triplet I). Neither triplet I nor triplet II were in perfect unison ($p < 0.0001$ for triplet I and $p < 0.0001$ for triplet II). In *Macaca*, only triplet I is more in unison than non-triplet pairs, however, neither triplet pair is perfectly in unison.

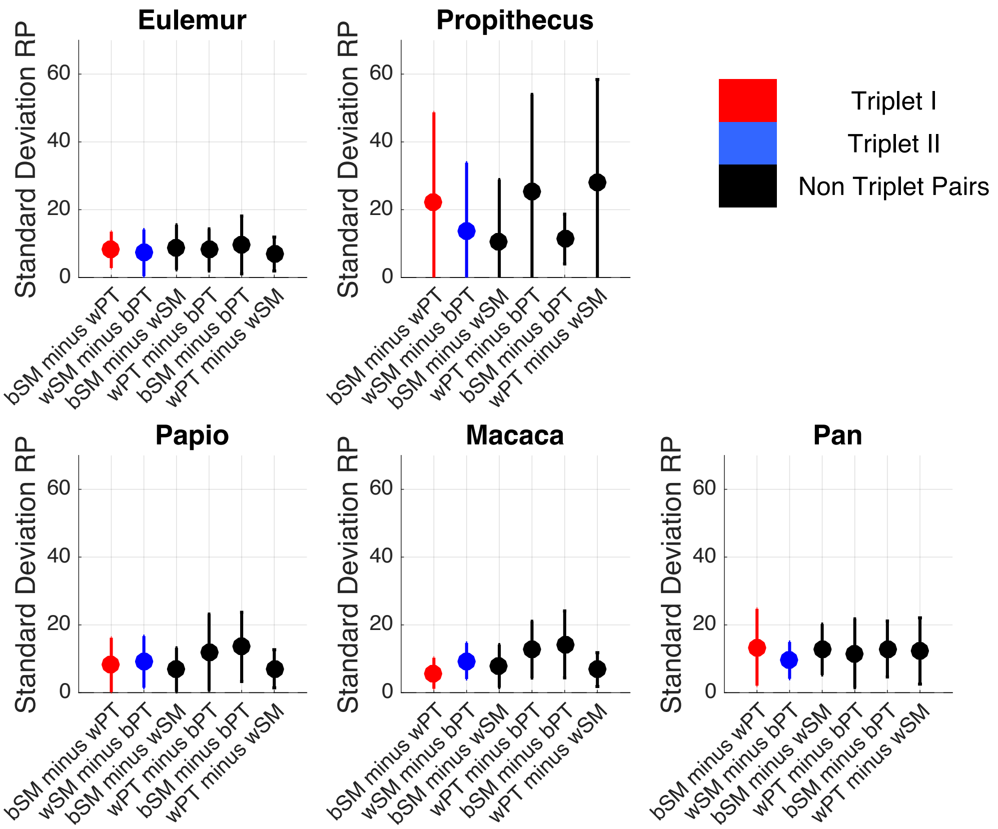
In *Pan*, both triplet I and triplet II had the lowest $\bar{\bar{\phi}}_{1-2}$ ($\bar{\bar{\phi}}_{1-2}$ = -7.36 ± 31.49° for triplet I, $\bar{\bar{\phi}}_{1-2}$= -13.22 ± 28.65° for triplet II). But neither triplet I nor triplet II had a $|\bar{\bar{\phi}}_{1-2}|$ distribution that was significantly lower than that of non-triplet muscles ($p = 0.3829$ in triplet I and $p = 0.4357$ for triplet II). Triplet I but not triplet II had a $\bar{\bar{\phi}}_{1-2}$ that was not significantly different from 0° ($p = 0.1824$ for triplet I and $p = 0.0111$ for triplet II). Thus, while triplet I may be active in unison in *Pan*, neither triplet I nor triplet II is more in unison than non-triplet muscles.


Are muscles in the same triplet more in unison than muscles in two different triplets?

	Triplet I	Triplet II	Non Triplet Pairs			
	bSM - wPT	wSM - bPT	bSM - wSM	wPT - bPT	bSM - bPT	wPT - wSM
Eulemur	5.68 ± 22.10°**	2.73 ± 21.43°*	20.38 ± 22.46°	17.43 ± 21.98°	23.11 ± 30.79°	14.70 ± 9.34°
Propithecus	23.28 ± 54.10°	14.56 ± 39.76°*	29.58 ± 38.41°	20.87 ± 52.56°	44.14 ± 19.82°	6.30 ± 70.13°
Papio	6.93 ± 15.30°*	40.84 ± 29.65°	24.01 ± 12.46°	57.92 ± 33.08°	64.85 ± 35.52°	17.07 ± 13.38°
Macaca	5.78 ± 20.70°*	30.96 ± 19.00°*	23.46 ± 19.26°	48.63 ± 24.98°	54.42 ± 28.50°	17.67 ± 16.63°
Pan	-7.36 ± 31.49°	-13.22 ± 28.65°*	16.15 ± 24.76°	10.28 ± 30.02°*	2.93 ± 26.66°*	23.50 ± 32.09°

Figure 3.3. The $\bar{\bar{\phi}}_{1-2}$ for all cycles that follow the triplet motor pattern. To determine whether muscles within the same triplet are more in unison than muscles in two different triplets, we calculated the $\bar{\phi}_{1-2}$ between muscle pairs for individual cycles, then took the $\bar{\bar{\phi}}_{1-2}$ across all cycles. A positive $\bar{\bar{\phi}}_{1-2}$ means that the muscle listed first fired before the second muscle. A negative $\bar{\bar{\phi}}_{1-2}$ means that the muscle listed second became active first. A red asterisk indicates that the distribution of $|\bar{\phi}_{1-2}|$ for the triplet muscle pair is significantly lower than that of non-triplet pairs ($p < 0.05$). A black asterisk indicates that the $\bar{\bar{\phi}}_{1-2}$ of the triplet pair is not significantly different from 0° ($p > 0.05$).

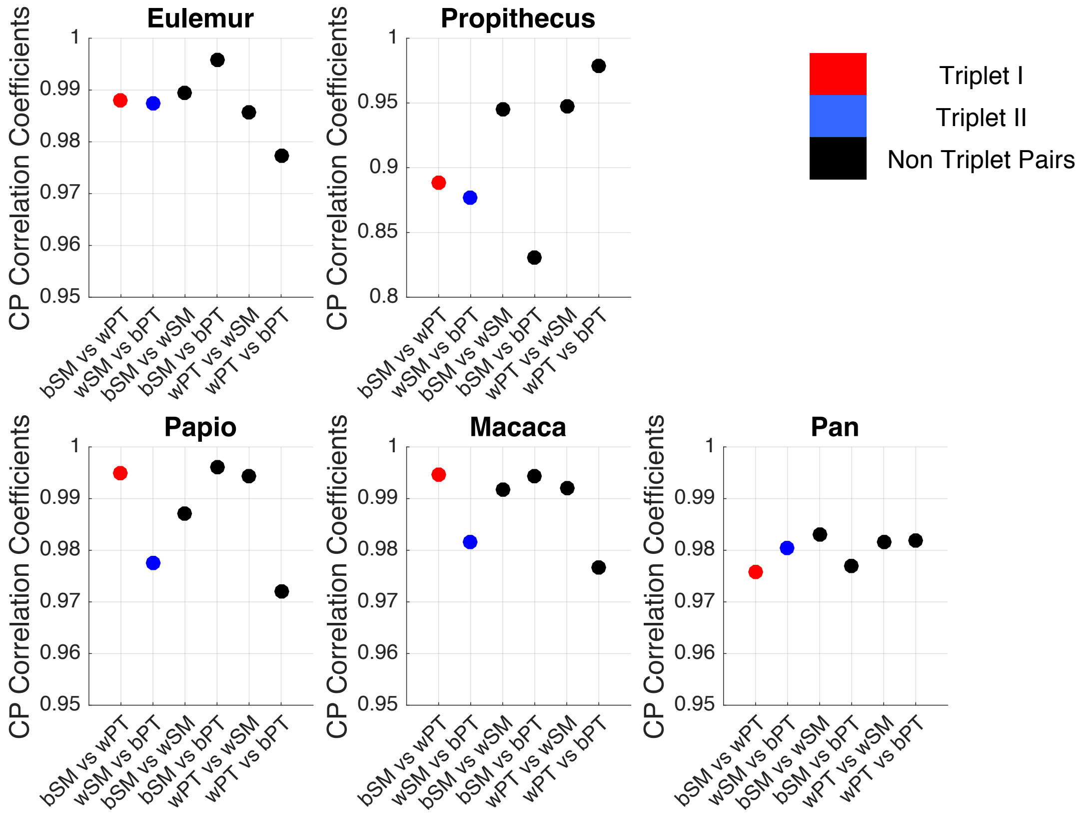
Are muscles in the same triplet more synchronized than muscles in two different triplets?



	Triplet I	Triplet II	Non Triplet Pairs			
	bSM - wPT	**wSM - bPT**	**bSM - wSM**	**wPT - bPT**	**bSM - bPT**	**wPT - wSM**
Eulemur	8.15 ± 5.38°	7.26 ± 6.88°	8.90 ± 6.65°	8.13 ± 6.22°	9.60 ± 8.52°	6.92 ± 5.00°
Propithecus	22.21 ± 26.52°	13.57 ± 20.30°	10.55 ± 18.34°	25.27 ± 28.74°	11.33 ± 7.34°	28.23 ± 30.17°
Papio	8.20 ± 8.02°	9.09 ± 7.61°	6.74 ± 6.50°	11.97 ± 11.23°	13.51 ± 10.21°	7.06 ± 5.62°
Macaca	5.73 ± 4.58°*	9.36 ± 5.33°*	7.83 ± 6.37°	12.70 ± 8.38°	14.25 ± 9.87°	6.81 ± 4.99°
Pan	13.40 ± 11.34°	9.53 ± 5.43°*	12.74 ± 7.52°	11.63 ± 10.16°	12.89 ± 8.25°	12.31± 9.77°

Figure 3.4. The $\bar{\sigma}_{\bar{\Phi}_{1-2}}$ for all triplet cycles. To determine whether muscles within the same triplet are more synchronized than muscles in two different triplets, we calculated $\sigma_{\bar{\Phi}_{1-2}}$ for all muscle pairs in a cycle. We then calculated $\bar{\sigma}_{\bar{\Phi}_{1-2}}$ for all triplet cycles. We used a two-way t-test to determine if $\bar{\sigma}_{\bar{\Phi}_{1-2}}$ for muscles within the same triplet is lower than the $\bar{\sigma}_{\bar{\Phi}_{1-2}}$ for muscles in two different triplets. A one-tailed two-way t-test compared the distribution of $\sigma_{\bar{\Phi}_{1-2}}$ of triplet muscles to that of non-triplet muscles. Values that are significantly less than non triplet pairs is indicated by a black asterisk ($p < 0.05$)

Are muscles in the same triplet more coordinated than muscles in two different triplets?



	Triplet I	Triplet II	Non Triplet Pairs			
	bSM - wPT	wSM - bPT	bSM - wSM	wPT - bPT	bSM - bPT	wPT - wSM
Eulemur	0.9880	0.9874	0.9896	0.9957	0.9856	0.9973
Propithecus	0.8888	0.8766	0.9446	0.8303	0.9469	0.9786
Papio	0.9950	0.9776	0.9871	0.9962	0.9945	0.9720
Macaca	0.9947	0.9817	0.9918	0.9943	0.9921	0.9766
Pan	0.9757	0.9803	0.9832	0.9770	0.9815	0.9819

Figure 3.5. The $\rho(\phi_1, \phi_2)$ for all triplet cycles. To determine whether muscles within the same triplet are more coordinated than muscles in two different triplets, we calculated $\rho(\phi_1, \phi_2)$ for all muscle pairs across all triplet cycles.

Synchrony

In *Macaca,* triplet I but not triplet II had a lower $\bar{\sigma}_{\phi_{1-2}}$ than non-triplet muscle pairs. However based on the one-sided two-way t-test, both triplet I and triplet II have $\bar{\sigma}_{\phi_{1-2}}$distributions that are significantly lower than non-triplet muscles ($p < 0.0001$ for triplet I $p = 0.0128$ for triplet II). Thus in *Macaca*, triplet I but not triplet II is more synchronized than non-triplet muscles. In Pan, triplet II but not triplet I is more synchronized than non-triplet muscles ($\bar{\sigma}_{\phi_{1-2}}$= 9.53 ± 5.43° $p = 0.0128$). In the other three species studied, both triplet I and triplet II did not have a $\bar{\sigma}_{\phi_{1-2}}$ that was lower than the $\bar{\sigma}_{\phi_{1-2}}$ of non-triplet muscles.

Coordination

Figure 4 shows the $\rho(\phi_1, \phi_2)$ for the six jaw elevator muscle pairs across all triplet cycles. In *Eulemur, Papio, Macaca,* and *Pan*, the six jaw elevator muscle pairs have $\rho(\phi_1, \phi_2)$ ranging from 0.97270 to 0.9962. In *Pan* Triplet II is more coordinated than all other muscle pairs; (ϕ_{wSM}, ϕ_{bPT}) is higher than all other $\rho(\phi_1, \phi_2)$ by 0.0013 to 0.0075 but triplet I is less coordinated than all other triplet pairs ($\rho(\phi_{bSM}, \phi_{wPT}) = 0.9757$). In *Macaca* triplet I is more coordinated than all other muscle pairs; $(\phi_{bSM}, \phi_{wPT}) = 0.9947$ is higher that all other $\rho(\phi_1, \phi_2)$ by 0.0004 to 0.0181. In all other species, the $\rho(\phi_1, \phi_2)$ of triplet muscle pairs is neither the highest nor the lowest $\rho(\phi_1, \phi_2)$. *Propithecus* has the most variability in jaw muscle coordination across the six jaw elevators; *Propithecus* $\rho(\phi_1, \phi_2)$ range from 0.8303 to 0.9786. Triplet I muscle pairs are more coordinated than triplet II muscle pairs in all species except *Pan*.

To get a more thorough understanding of the degree to which triplet muscles are coordinated compared to non-triplet muscle pairs, Figures 5-8 show the distribution of $\rho(\phi_1, \phi_2)$ for triplet and non-triplet pairs in triplet and non-triplet cycles. Two-way t-tests were used to identify significant differences ($p \leq 0.05$).

In *Eulemur* triplet muscle pairs have a lower mean $\rho(\phi_1, \phi_2)$ and standard deviation in $\rho(\phi_1, \phi_2)$ during triplet cycles compared to non-triplet cycles ($p = 0.004$): triplet muscles are more coordinated during triplet cycles than during non-triplet cycles (Figure 5). However for non-triplet muscle pairs neither the mean $\rho(\phi_1, \phi_2)$ nor the standard deviation in $\rho(\phi_1, \phi_2)$ differs significantly between triplet and non-triplet cycles. Unlike *Eulemur*, there are no significant changes in the coordination of *Propithecus* triplet muscles during triplet and non-triplet cycles (Figure 6).

In *Papio,* there are no significant differences in the coordination of triplet muscles between triplet and non-triplet cycles, but the non-triplet muscles are significantly more coordinated during non-triplet cycles than in triplet cycles ($p = 0.043$). Moreover, during triplet cycles, the mean $\rho(\phi_1, \phi_2)$ of triplet muscles is significantly higher than non-triplet muscles ($p = 0.007$) and the standard deviation in $\rho(\phi_1, \phi_2)$ for non-triplet muscles is double that of triplet muscles. During non-triplet cycles there are no significant differences between triplet and on-triplet muscles in $\rho(\phi_1, \phi_2)$ ($p = 0.976$). Thus, in *Papio* non-triplet muscles are more coordinated during non-triplet cycles than during triplet cycles and triplet muscles are more coordinated than non-triplet muscles during triplet cycles.

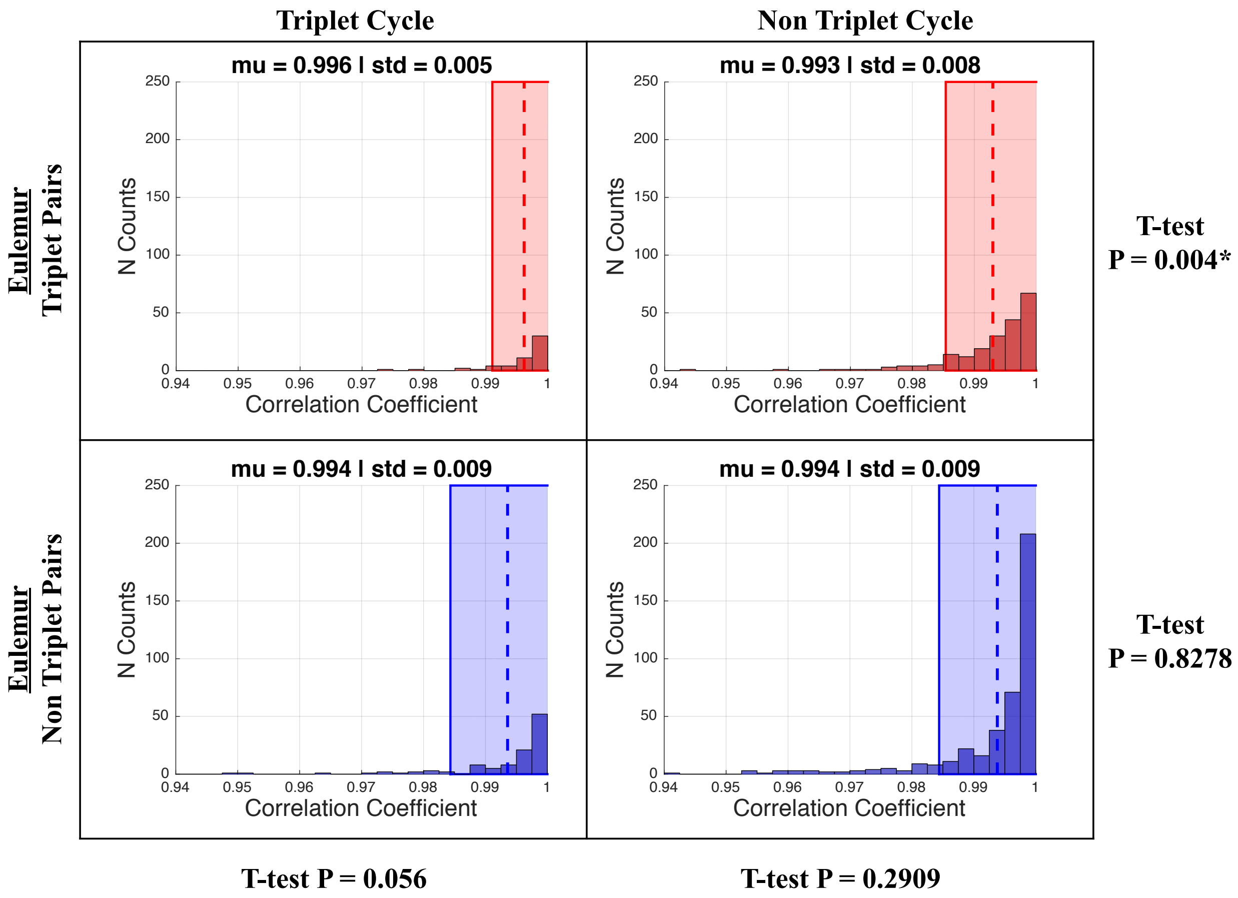


Figure 3.6. **Eulemur** $\rho(\phi_1, \phi_2)$ this figure compares the distribution of $\rho(\phi_1, \phi_2)$ for muscles in the same triplet to that of muscles in two different triplets for both triplet and non-triplet cycles. A two-way t-test was conducted to determine whether the distribution of $\rho(\phi_1, \phi_2)$ was significantly different between conditions.

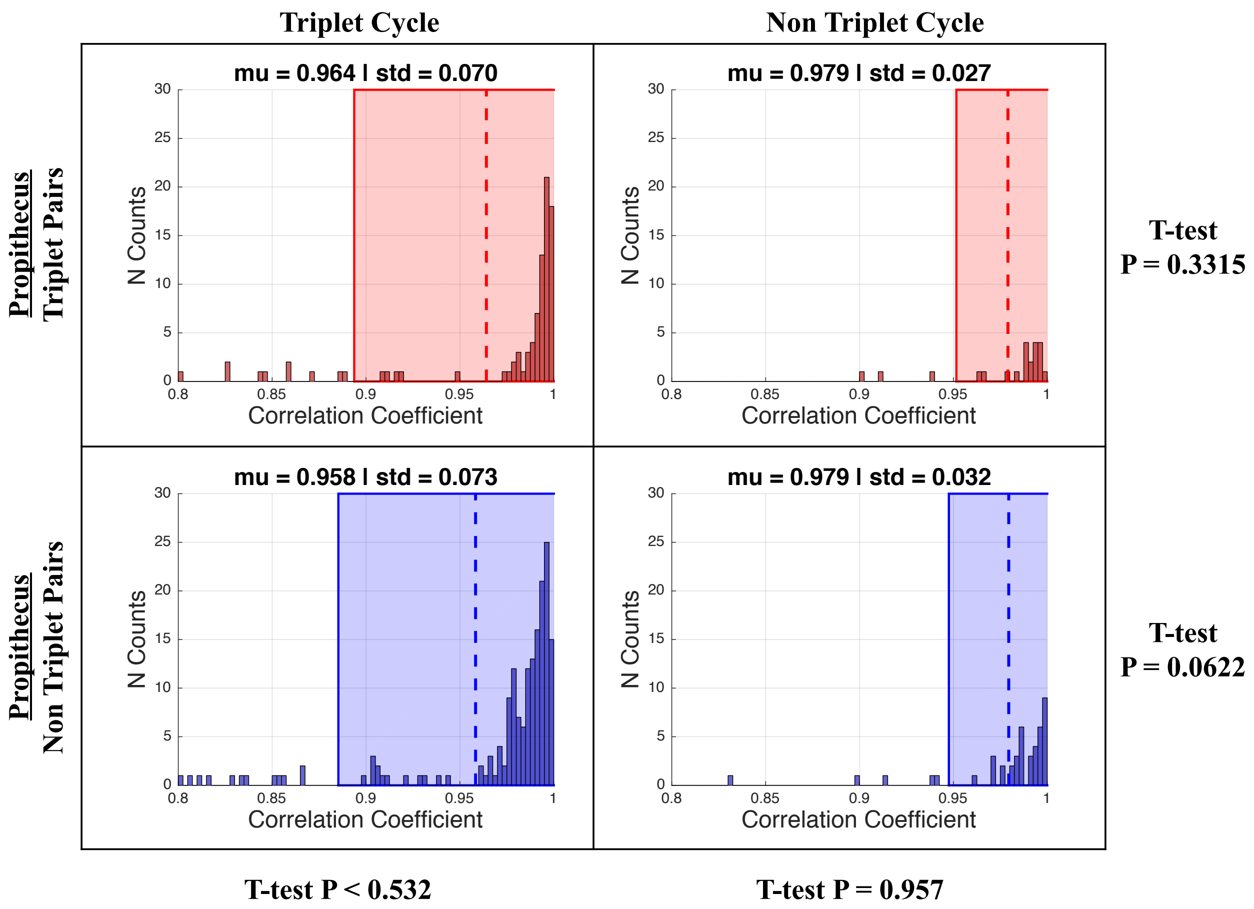


Figure 3.7. ***Propithecus*** $\rho(\phi_1, \phi_2)$ this figure compares the distribution of $\rho(\phi_1, \phi_2)$ for muscles in the same triplet to that of muscles in two different triplets for both triplet and non-triplet cycles. A two-way t-test was conducted to determine whether the distribution of $\rho(\phi_1, \phi_2)$ was significantly different between conditions.

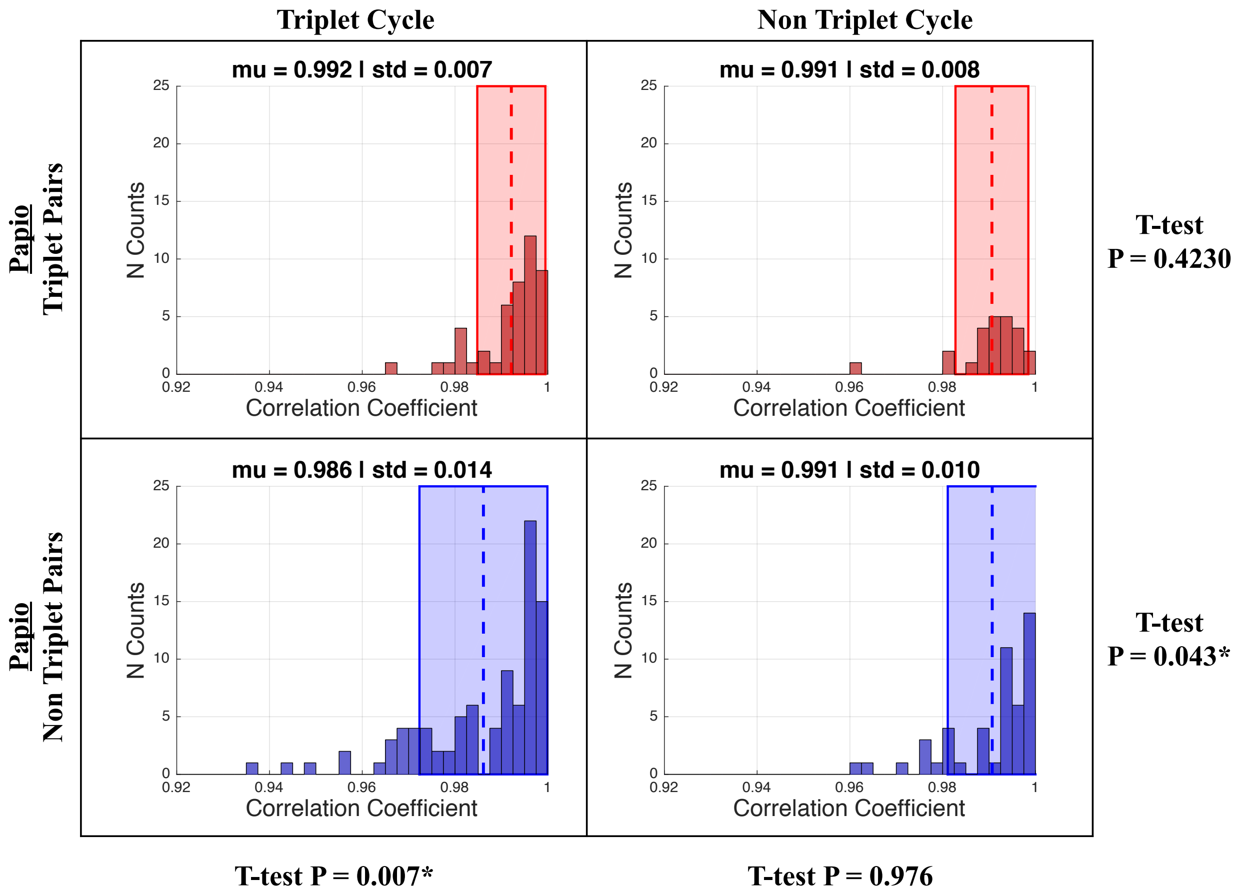


Figure 3.8. **Papio** $\rho(\phi_1, \phi_2)$ this figure compares the distribution of $\rho(\phi_1, \phi_2)$ for muscles in the same triplet to that of muscles in two different triplets for both triplet and non-triplet cycles. A two-way t-test was conducted to determine whether the distribution of $\rho(\phi_1, \phi_2)$ was significantly different between conditions.

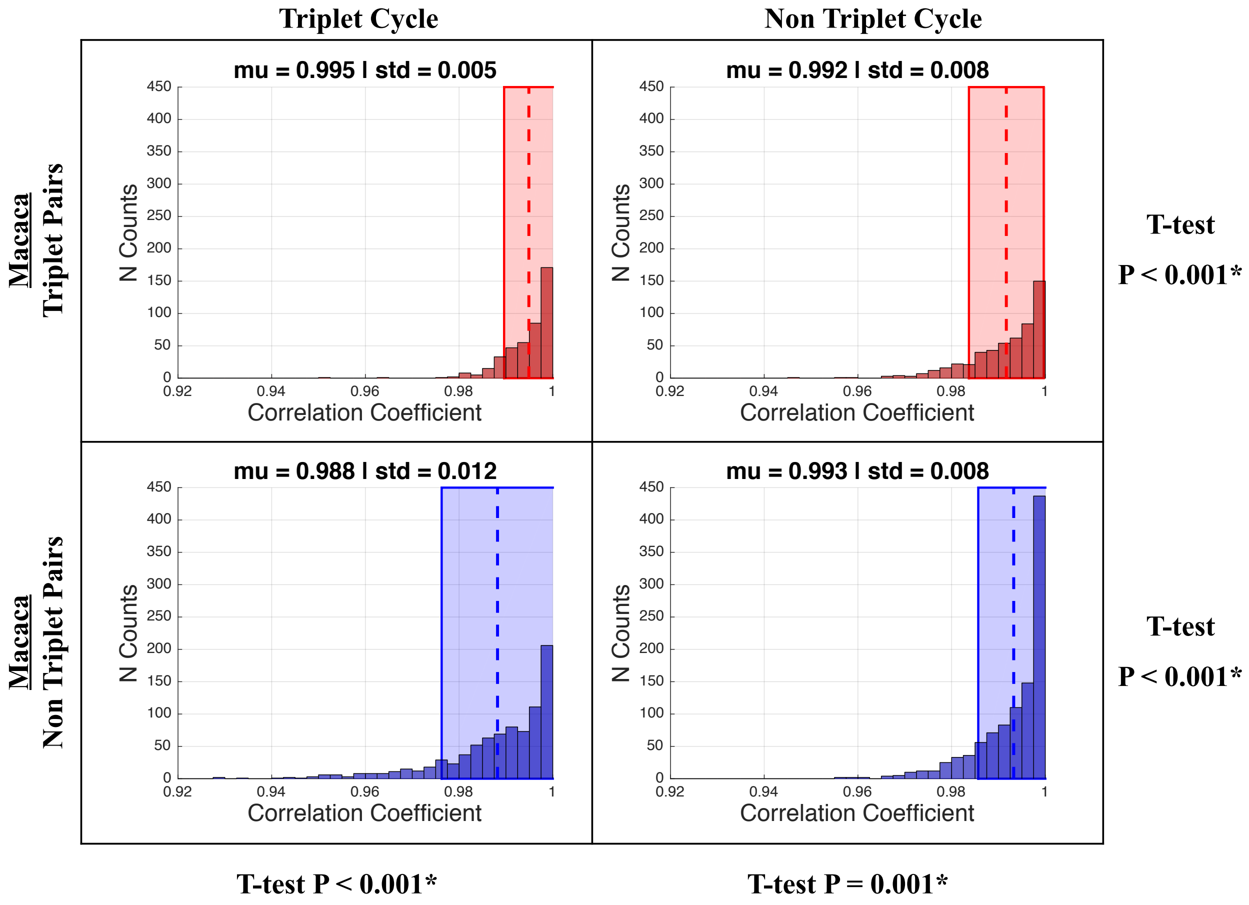


Figure 3.9. ***Macaca*** $\rho(\phi_1, \phi_2)$ this figure compares the distribution of $\rho(\phi_1, \phi_2)$ for muscles in the same triplet to that of muscles in two different triplets for both triplet and non-triplet cycles. A two-way t-test was conducted to determine whether the distribution of $\rho(\phi_1, \phi_2)$ was significantly different between conditions.

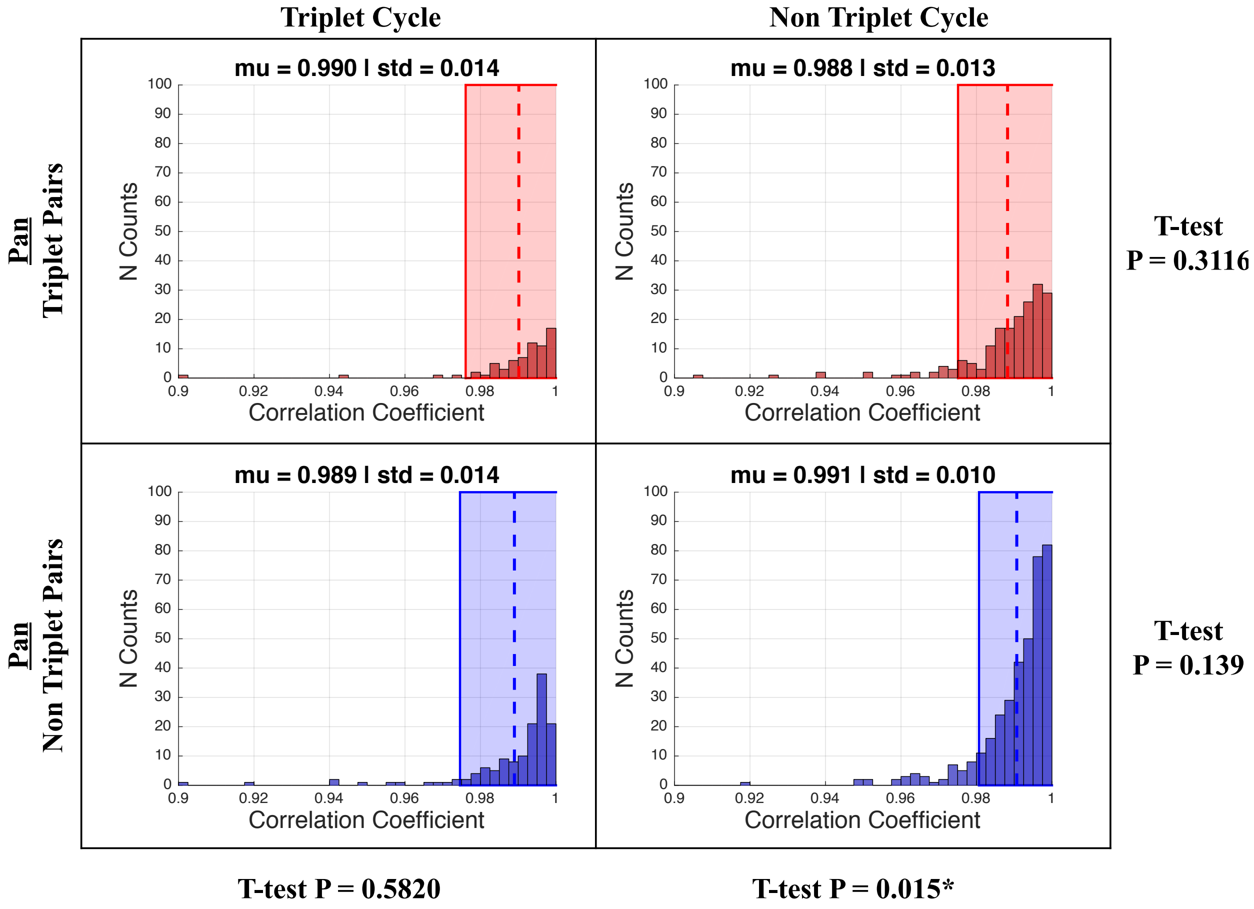


Figure 3.10. **Pan** $\rho(\phi_1, \phi_2)$ this figure compares the distribution of $\rho(\phi_1, \phi_2)$ for muscles in the same triplet to that of muscles in two different triplets for both triplet and non-triplet cycles. A two-way t-test was conducted to determine whether the distribution of $\rho(\phi_1, \phi_2)$ was significantly different between conditions.

In *Macaca* triplet muscles are more coordinated during triplet cycles than in non-triplet cycles ($p < 0.001$), and they have both a lower mean $\rho(\phi_1, \phi_2)$ and higher standard deviation in $\rho(\phi_1, \phi_2)$ during non-triplet cycles. Non-triplet muscles are more coordinated during non-triplet cycles than triplet cycles ($p < 0.001$), and they have both a lower mean $\rho(\phi_1, \phi_2)$ and a higher standard deviation $\rho(\phi_1, \phi_2)$ during triplet cycles.

In *Pan,* the triplet muscles are more coordinated during triplet cycles than in non-triplet cycles, but this difference is not significant. Similarly, the non-triplet muscles are more coordinated during non-triplet cycles than during triplet cycles, but the differences are not significant. In *Pan* non-triplet muscles are significantly more coordinated than triplet muscles ($p = 0.015$) during non-triplet cycles.

DISCUSSION

In this study, succinct, quantifiable definitions of unison, synchrony, and coordination are proposed and used to test hypotheses regarding the role the CNS plays in modulating the relative timing and amplitude of muscle activity. We hope that these definitions and the variables used to quantify them will, by formalizing future discussions, help to advance studies of coordination and synchronization in musculoskeletal biomechanics and motor control. Application of these definitions and techniques to muscle triplets in the feeding systems of primates suggests that muscle triplets are in unison, synchronized and coordinated to varying degrees in different species. Triplet I muscles are more in unison than all other muscle pairs in *Eulemur, Macaca,* and *Papio.* However, triplet muscle pairs are mostly not more tightly synchronized than non-triplet pairs. The only exceptions to this are *Macaca*, in which triplet I muscles are more tightly synchronized than other pairs and *Pan*, where triplet II muscles are more tightly synchronized than other pairs. Thus, triplet muscles exhibit more similar activity times in some species than others, but they are mostly not more tightly synchronized than other muscles. However, all species showed higher coordination between triplet muscles during triplet cycles than in non-triplet cycles, although this difference was only significant in *Macaca* and *Eulemur*. Similarly, all species showed lower levels of coordination in non-triplet muscles during triplet cycles, although the difference was significant in only two species. Four species showed higher coordination and decreased variation in non-triplet muscles during non-triplet cycles, with these differences significant in two species. Together these results suggest that triplet muscles are more highly coordinated during triplet cycles and non-triplet muscles are more tightly coordinated during non-triplet cycles, suggesting that different patterns of coordination predominate in different cycles.

Relative phase has been used in multiple fields of study to quantify the interaction between two cyclic signales. Here, this measure was used to quantify the degree of co-modulation between muscle pairs. We proposed succinct and quantifiable definitions of unison, synchrony, and coordination that can be widely used to describe the interaction or degree of co-modulation between any two entities that repeatedly move in a stereotyped manner. This includes muscle pairs, joints (e.g. the shoulder and the wrist during reach and grasp), kinematic markers, and even neural populations. Relative phase assumes that the signal is sinusoidal or unimodal. For example, if a muscle bursts twice within a single cycle, it will accumulate 720° of continuous phase as oppose to 360°. In the present study, we eliminated all muscle activity prior to onset and after offset to make the signal more sinusoidal and ensure that each muscle accumulates approximately 360° per cycle.

Implications for jaw elevator motor control in primates

The fact that triplets are not characterized by stereotyped and hard-wired patterns of activation means that triplet motor patterns are not *invariant* units of control for the CNS (Overduin et al. 2008). Rather, they appear to be flexibly recruited in some cycles and not others. Moreover, the fact that triplet muscles are more coordinated during triplet cycles compared to non-triplet cycles but they are not more tightly synchronized suggests that active modulation may be functionally important during some *times* in the cycle but not others. Triplet I but not Triplet II muscles have the lowest temporal offset in *Papio* and *Macaca*, while *Eulemur* triplet muscles are in unison. In these three species, at least triplet I muscles may be in unison and coordinated but not synchronized. However, triplet II is neither in unison nor coordinated in any of the species studied. These findings suggest that triplet II, which is recruited later in the cycle, during the slow close phase, is more variable than triplet I, which is recruited earlier in the cycle, during the fast close phase (Ross and Iriarte-Diaz, 2014). We hypothesize that the triplet motor pattern may be functionally significant earlier in the masticatory cycle, as the jaw moves into the power stroke, but may not be maintained throughout the cycle, leading to low synchrony between muscles of the same triplet and increased variability in triplet II.

A different pattern of coordination that does not require triplet muscles appears to be utilized during non-triplet cycles, leading to increased coordination between non-triplet muscle pairs in *Propithecus, Papio, Macaca,* and *Pan.* These findings suggest that non-triplet motor patterns are recruited at least as frequently as the triplet motor pattern, but the intrinsic criteria used by the system to assess which motor pattern to recruit are unknown. Further EMG studies should be aimed at defining the full range of jaw elevator motor patterns and their relationship to jaw kinematics and the stages of the chewing sequence with the hope of uncovering the extent to which various optimality criteria affect the system (Ross and Iriarte-Diaz 2014) and the neural factors that may influence specific muscle activation patterns (Mussa-Ivaldi et al. 1990).

The fact that triplet I muscles are active in unison and coordinated suggests that they may form a *movement primitive* or module in *Eulemur, Macaca* and *Papio*. Movement primitives have been proposed as a solution to the “ill posed” problem (Mussa-Ivaldi et al. 1990) which, in the feeding system, is the problem that for any given movement there is an infinite combination of muscle activation strategies that would produce the observed or desired kinematics (Vinyard et al 2008; Mussa-Ivaldi et al. 1990). It is hypothesized that movement primitives provide a mechanism for the CNS to bootstrap complex problems to identify optimal patterns of muscle activation.

Unlike triplet I, triplet II is neither synchronized nor coordinated in any of the species studied suggesting that the CNS regulates triplet I more tightly than it does triplet II. One way this might be done, according to the uncontrolled manifold hypothesis, is for the CNS to identify and optimize only task relevant variables. Variability that does not affect the behavioral goal is allowed since correcting task irrelevant variability is an unnecessary waste of effort (Tresch and Jarc 2009).

From the neural and motor control perspectives, motor primitives can serve one of two functions: They may be a constraint on movement control; or they may be an optimization strategy (Nazarpour et al. 2012). The first case implies that motor patterns are conserved due to hard-wired neural circuitry. The second scenario implies that because any movement of a body part is influenced by similar biomechanical principles, the optimal muscle synergies and/or motor primitives will be broadly conserved (Nazarpour et al. 2012). If conservation in motor patterns is a result of optimization, then any repertoire of muscle synergies and /or motor primitives stored in the CNS would serve as a reserve of optimal shortcuts as opposed to neural constraints on behavior.

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GLOSSARY

	Metric	Abbreviation	Equation
	Continuous phase	ϕ_1	$H(t) = \frac{1}{\pi}\int_{-\infty}^{\infty} \frac{u(\boldsymbol{\tau})}{(t-\tau)}\, d\tau$ $\phi = arctan\left(\frac{real(H(t))}{imaginary(h(t))}\right)$
	Relative phase (RP)	ϕ_{1-2}	$\phi_1 - \phi_2$
Unison	**Mean RP** (Single cycle)	$\bar{\phi}_{1-2}$	$\bar{\phi}_{1-2} = \frac{\sum_1^{100} \phi_{1-2}}{100}$
	Grand mean RP (All cycles)	$\bar{\bar{\phi}}_{1-2}$	$\bar{\bar{\phi}}_{1-2} = \frac{\sum_1^N \bar{\phi}_{1-2}}{N}$ N = number of cycles
	Standard deviation in grand mean RP	$\sigma_{\bar{\bar{\bar{\phi}}}_{1-2}}$	$\sigma_{\bar{\bar{\bar{\phi}}}_{1-2}} = \sqrt{\frac{\sum_1^N(\bar{\phi}_{1-2} - \bar{\bar{\phi}}_{1-2})^2}{N-1}}$
Synchrony	**Standard deviation in RP** (Single cycle)	$\sigma_{\phi_{1-2}}$	$\sigma_{\bar{\bar{\bar{\phi}}}_{1-2}} = \sqrt{\frac{\sum_1^{100}(\phi_{1-2} - \bar{\phi}_{1-2})^2}{99}}$
	Mean standard deviation RP (All cycles)	$\bar{\sigma}_{\phi_{1-2}}$	$\bar{\sigma}_{\bar{\phi}_{1-2}} = \frac{\sum_1^N \sigma_{\bar{\phi}_{1-2}}}{N}$

	Standard deviation in standard deviation RP	$\sigma_{\bar{\sigma}_{\phi_{1-2}}}$	$\sigma_{\bar{\sigma}_{\bar{\phi}_{1-2}}} = \sqrt{\frac{\sum_1^N(\sigma_{\bar{\phi}_{1-2}} - \bar{\sigma}_{\bar{\phi}_{1-2}})^2}{N-1}}$
Coordination	**Correlation Coefficient**	$\rho(\phi_1, \phi_2)$	$\rho(\phi_1, \phi_2) = \frac{1}{N-1}\sum_1^N\left(\frac{\phi_1 - \phi_1}{\sigma_{\phi_1}}\right)\left(\frac{\phi_1 - \phi_1}{\sigma_{\phi_2}}\right)$

REPRESENTATION OF OROFACIAL BEHAVIORS IN THE PRIMARY MOTOR CORTEX OF *MACACA MULATTA*

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ABSTRACT

Huang et al. (1988) observed that representations of the face, jaw, and tongue are organized medial to lateral (in that order) in the orofacial area of the primary motor cortex (MIo) with a large region of overlap between the three areas. However, the motor cortex is organized into functional zones that each represents ethologically relevant actions within a larger somatotopic gradient (Graziano 2002). The present study utilizes an intracortical microstimulation (ICMS) protocol developed by Park et al. (2001) to determine whether MIo is organized into clusters of behaviorally relevant groups of muscles within the larger somatotopic gradient described by Huang et al. (1988). This protocol increases the precision and specificity of ICMS by minimizing current spread and neural facilitation caused by either larger amplitudes or repetitive stimulation. We test the following predictions: (1) muscles innervated by cranial nerves have a faster response time than muscles innervated by anterior cervical rami; (2) excitatory responses have faster response times than inhibitory responses; (3) contralateral muscles elicit a greater response than ipsilateral muscles; (4) muscles with synergistic actions will be positively correlated while antagonistic muscles are negatively correlated (i.e. synergistic muscles will be recruited together while antagonistic muscles will be actively inhibited); and (5) groups of muscles that represent behaviorally relevant actions will be spatially clustered together. Our findings show that (1) muscles innervated by some cranial nerves do have faster response times than muscles innervated by anterior cervical rami, but this difference is not always significant; (2) excitatory muscle responses are not faster than inhibitory responses; (3) contralateral muscles do not respond significantly ($p < 0.05$) more than ipsilateral muscles; (4) muscles with synergistic actions are positively correlated and frequently recruited together. However, antagonistic muscles were also positively correlated producing modulated and possibly asymmetric actions.

(5) We successfully confirmed the somatotopic gradient described by Huang et al. (1988) and identified specific behaviorally relevant actions that are spatially clustered together in MIo. Moreover, one of the two clusters represents jaw depression and hyoid elevation/ protraction in both monkeys. Monkey H's clusters varied from those of monkey M in a potentially a use dependent manner. However differences in the ethological clusters in both monkeys may also be affected by array placement.

INTRODUCTION

Graziano and colleagues have suggested that cortical representation of behavior is influenced both by the structure of the body and common actions in the behavioral repertoire (Graziano 2016). They hypothesized that the cortex contains distinct functional zones, which represent ethologically relevant actions like chewing, lapping, or reach to grasp. Maps of distinct functional zones are generated using intracortical microstimulation (ICMS) to excite pyramidal tract neurons in layer V of the primary motor cortex (MI). Almost all of the studies that identify distinct functional zones that represent specific muscles have focused on the upper limbs; very little information is available regarding the orofacial system (Huang et al. 1988).

Huang et al. (1988) previously used ICMS to cortically map muscle representation in MIo. They found that face was represented most medially, followed by jaw, and finally the tongue was represented most laterally. They found that 85% of all jaw movements elicited were jaw opening and that jaw closing was represented in fewer loci. Most importantly, Huang et al. (1988) observed that many responses were "mixed" because responses from face, jaw, and/or tongue were elicited simultaneously and they recognized that a simple somatotopic view of the cortex did not capture the specific and complex musculotopy they observed. The present study confirms the somatotopic gradient observed by Huang et al. (1988) and identifies specific functional groups of muscles that are spatially clustered together in MIo.

The orofacial primary motor cortex (MIo) is uniquely qualified to elucidate how actions are represented within the cortex and the role primary motor cortex plays in generating behavior.

The monosynaptic corticobulbar projections from MIo to the trigeminal, facial, and hypoglossal motor nuclei allow researchers a direct look at how activity in MI relates to muscle activation (Kuypers 1958; Kuypers and Lawrence 1967; Nordstrom et al. 1999; Morecraft et al. 2001; Morecraft et al. 2014). A single hemisphere of MIo innervates both contralateral and ipsilateral motor neuron pools allowing for the differential control of each motor neuron pool to produce bilaterally coordinated behaviors (Nordstrom et al. 1999). The trigeminal, facial, and hypoglossal nuclei are organized musculotopically into subnuclei that each innervates a subset of muscles (Sokoloff and Deacon 1992; Morecraft et al. 2001; Sherwood et al. 2005). For example, physiological studies of the hypoglossal motor nucleus suggest that topographically segregated motor neuron pools correspond to a functional distinction between tongue protrusion and tongue retraction (Sokoloff and Deacon 1992). Multiple yet discrete microzones of orofacial regions have also been recognized in MIo and previous studies have shown that there are multiple representations of a single muscle in MIo (Huang et al. 1988; Morecraft et al. 2001). Morecraft et al. (2001) predicted that microzones in the cortex preferentially target specific subsectors of the corresponding motor nucleus controlling specific groups of orofacial muscles (Morecraft 2001). They suggested that different cortical orofacial representations preferentially influence specific groups of muscles. However, this hypothesis has never been specifically tested. In the present study, we use ICMS to test the hypothesis that discrete microzones of MIo preferentially influence specific functional groups of orofacial muscles.

Intracortical microstimulation (ICMS) artificially excites a small area of the cortex and induces a small number of muscle activation patterns. One criticism of ICMS is that it “hijacks” the cells and connections involved in movement production (Kutch and Valero-Cuevas 2012). However, the alternatives to ICMS also have their downfalls. Intracellular recordings can be used to understand how cortical firing is correlated to the activation of specific muscles. However, these studies are limited by the number of cortical connections that can be traced to the motor neuron and from the motor neuron to their target muscles; they generally reveal only cortico-motoneuronal connections. It is unable to affect connections that require interneurons, including al inhibitory connections and muscles innervated by anterior cervical rami (Lemon 1988; Park et al. 2004). Transcranial magnetic stimulation (TMS) and transcranial electrical stimulation (TES) have also been used in humans to study how cortical activity influences muscle activation. However, the extent of cortical activation with these methods is larger than ICMS and lacks precision (Park et al. 2004). The present study uses a protocol developed by Park et al. (2001 2004) to systematically map muscle representation in the MIo of macaques (*Macaca mulatta*) using ICMS. The protocol developed by Park et al. (2014 2004) utilizes single biphasic pulses at amplitudes and frequencies low enough to cause motor neuron discharge and produce an electromyographic (EMG) response without producing observable behavioral responses to the stimulation. Stimulus triggered averaging was used to identify the strength and latency of each muscle's response. Park et al.'s (2001 2004) protocol increases the precision and specificity of ICMS by minimizing current spread, and neural facilitation. In the present study, we use this method to test a series of hypothesis regarding the interaction between the primary motor cortex and orofacial muscles.

We hypothesize that MIo is organized into discrete microzones and the muscles that comprise each microzone within MIo are influenced both by the structure of the body and common actions in a specific individual's behavioral repertoire (Huang et al. 1988; Graziano 2016). We predict that functional groups of muscles are topographically segregated within MIo. Our findings suggest that behavior is organized into a discrete number of ethologically relevant actions within MIo and that the behaviors represented can potentially be modified in a use dependent manner.

Response time

The corticobulbar tract contains monosynaptic connections between MIo and the trigeminal, facial, and hypoglossal motor nuclei. These connections make largely excitatory connections to the motor neuron pools while inhibitory connections are routed via inhibitory interneurons in the medial bulbar reticular formation (Takatori et al. 1981). Muscles innervated by the anterior rami of C1-C3 are at least bisynaptic and have an additional synaptic connection in the anterior horn of the spinal cord. Thus, we predict that cranial muscles will have shorter response times than muscles innervated by anterior rami and that excitatory responses will have shorter response times than inhibitory responses.

Side preference

The corticobulbar tract results in both ipsilateral and contralateral projections to the motor neuron pools. In fact loss of unilateral corticobulbar input to the trigeminal (V) motor nucleus produces no discernable deficits in behavior because the muscles of mastication receive bilateral inputs. However loss of unilateral corticobulbar input to the facial (VII) and hypoglossal (XII) motor nuclei result in contralateral motor deficits including loss of expression, and projection of the tongue towards the side of the lesion (Mason 2011). Huang et al (1988) also elicited bilateral muscle twitches when MIo was stimulated with ICMS. However, they observed that contralateral responses were predominant. Thus, while we expect to see bilateral muscle responses, we predict that there will be more responses from contralateral muscles than ipsilateral muscles.

Global correlations

If MIo were arranged into functionally relevant actions, we would expect muscles with synergistic actions to be activated together. We predict that the response strengths of synergistic muscles will be positively correlated while the response strengths of antagonistic muscles will be negatively coordinated. Antagonistic muscles will be inhibited while protagonist muscles are active and vice versa.

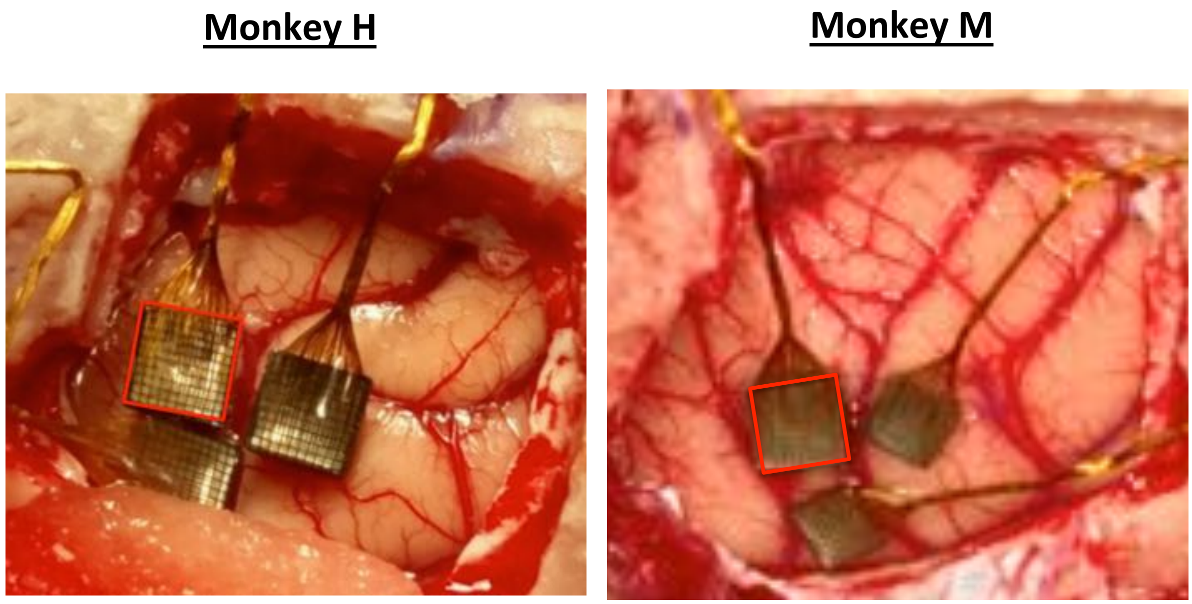
Ethological/ Musculotopic organization

Additionally, Huang et al. (1988) observed that MIo is divided into discrete zones of representation. Jaw muscles were represented medially in a horseshoe configuration around a more laterally located tongue representation. Moreover they observed larger proportions of jaw depression and little to no representation of jaw elevation. We expect to see similar motifs in representation across MIo with jaw muscles represented more medially compared to tongue and hyoid musculature. Additionally we expect to see larger proportions of jaw depression and tongue movement with little to no representation of jaw elevation.

METHODS AND MATERIALS

All surgical and behavior procedures were approved by the University of Chicago IACUC and conform to the principles outlined in the Guide for the Care and Use of Laboratory

Animals. Before, during, and after experimentation, monkeys were assessed regularly for general well-being.



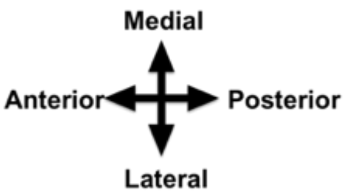


Figure 4.1 Positioning of the array in monkey H's and monkey M's MIo. The MIo array for both monkeys is shown inside a red box

Neural arrays

Under general anesthesia, each monkey was chronically implanted with two silicon-based microelectrode arrays in the orofacial region of MI in the left hemisphere (figure 1) (1.5 mm in length, 10 x 10 grid, 400 µm inter-electrode spacing, Blackrock Microsystems, Utah, USA,). Microelectrode tips were coated with iridium oxide. Implantation sites were verified based on surface landmarks and observed evoked responses from the tongue and fingers after monopolar surface stimulation of MIo (50 Hz, 200 µs pulse duration, 2–5 mA) during the surgical procedure. During each recording session, signals were amplified (gain 5000×); bandpass filtered

(0.25–7.5 kHz), and recorded digitally (14-bit, 30 kHz) using the Grapevine NIP acquisition system (Ripple Microsystems). Both monkeys' arrays were implanted in the left hemisphere of MIo.

Electromyography (EMGs)

At least two weeks after the array implantation, each monkey was sedated using general anesthesia and was chronically implanted with bipolar EMGs. The EMGs were sutured into place in the muscles listed below (table 1). All EMG wires were tunneled beneath the skin to the right posterior rostral portion of the skull. All EMG leads converged onto a 28 Position Male Nano-Miniature Circular Omnetics Connector (Omnetics Connector Corporation, Minnesota, USA). The Omnetics connector was secured in a hollow titanium holder, which was secured with four 6-7 mm bone screws. During each recording session, the EMG signals were collected using Grass Technologies amplifier LT15.

Table 4.1 EMG Muscles

Muscle	**Abbreviation**	**Action**	**Side**	**Monkey H**	**Monkey M**
Superficial Masseter	Sup Mass	Elevate mandible	L	x	x
			R		
Anterior Digastric	Ant Dig	Depress mandible Elevate hyoid	L	x	x
			R	x	x
Geniohyoid	Geniohy	Elevate and protract hyoid Depress mandible	L	x	x
			R	x	x

Posterior Mylohyoid	Pos Mylohy	Elevate hyoid and tongue	L	x	x
			R	x	x
Sternohyoid	Sternohy	Depress hyoid	L	x	x
			R	x	x
Styloglossus	Styloglos	Elevate and retract tongue	L	x	
			R	x	
Hyoglossus	Hyoglos	Depress and retract tongue	L		x
			R		x
Genioglossus	Genioglos	Protrude tongue	L	x	x
			R	x	x

Data collection

Each recording session lasted approximately two to three hours during which time the monkey sat still without performing any tasks. The monkey was seated in a plexiglass chair and was head fixed such that the monkey's head was completely immobilized. Each monkey had freedom of movement from the shoulders down, however, they were trained to sit still for extended periods of time daily for two continuous months prior to data collection. Each unit in the neural array was stimulated 2000 times for monkey H and 1500 times for monkey M continuously at 28 µA amplitude and a frequency of 15 Hz for monkey H and 15-40 uA at14 Hz for Monkey M (figure 2). In Monkey M, some cells required lower stimulus amplitudes than others to evoke an EMG response without evoking behavior. EMG signals were sampled at a frequency of 30 kHz during stimulation.

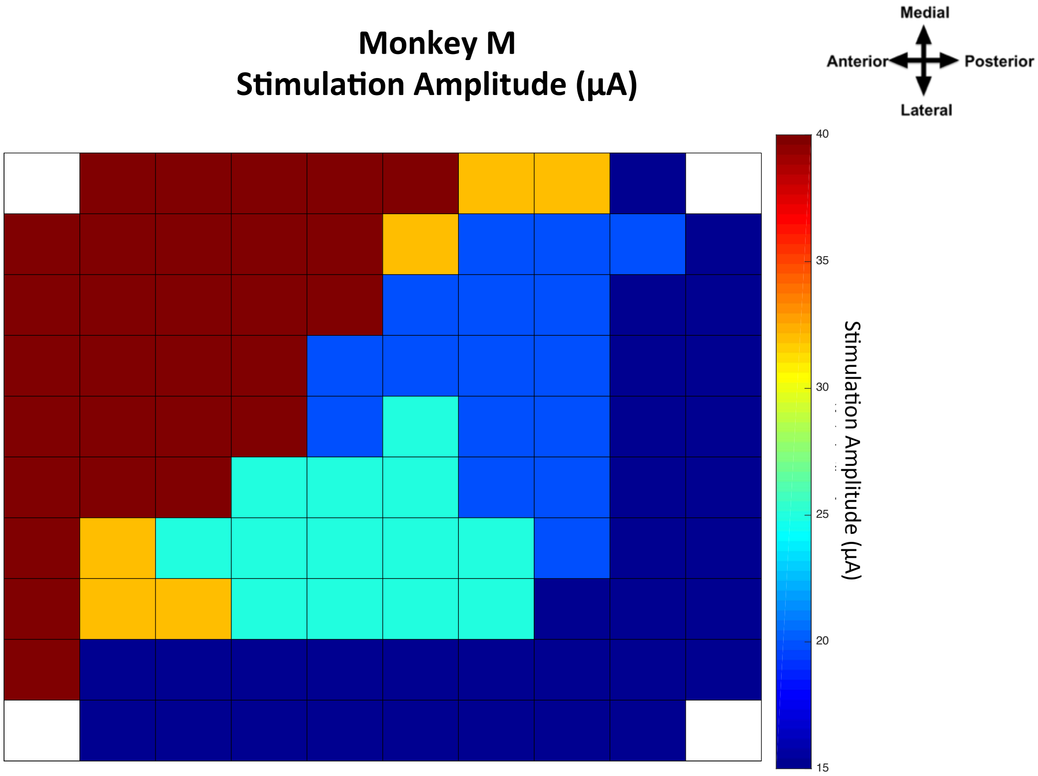


Figure 4.2. Monkey M's electrodes were stimulated at a range of amplitudes ranging from 15-40 uA.

Preprocessing

The raw EMG data for each stimulation event were extracted over a 60 ms time period (20 ms before the stimulation to 40 ms after stimulation) using the Neuroshare software package for Matlab (neuroshare.org). The EMG response for all 13 muscles was rectified and the stimulus-triggered average was calculated for all stimulations. All EMG responses were z-scored based on the mean and standard deviation in EMG signal from -20 ms to 12.5 ms prior to stimulation. EMG responses to stimulation had to fit two criteria (1) occur at least 5 ms after stimulation and (2) exceed either a z-score of 2 or -2 for 2 ms continuously. Response onset time and extrema response strength were recorded for each of the 13 EMG channels for all electrodes stimulated.

Global Correlations

Extrema responses were replaced with a +1 for activation (positive z-score), or -1 for inhibition (negative z-score). Cross correlations between muscles were then calculated at zero lag time.

K-means Clustering

The simplified muscle responses (+1 for activation and -1 for inhibition) were used to determine optimal clustering. The Kalinski-Harabaz criterion was used to compare the variation in muscle responses within a cluster to muscle responses between different clusters and identify the optimal number of clusters to be used in k-means clustering. K-means clustering was then utilized to sort electrodes into clusters based on the extrema muscle responses elicited. The mean z-scored muscle response strength and standard deviation in z-scored response strength were calculated for all the electrodes in each cluster.

RESULTS

Stimulus strength

All electrodes were stimulated at 28 μA in monkey H. However, in monkey M, the amplitude of stimulation had to modified based on the location of the electrode. Some electrodes produced observable twitches and so the amplitude had to be reduced. No observable behavior was produced even with 40 μA of stimulation in the anterior medial corner of monkey M's array, however the electrodes became increasingly sensitive to lower amplitudes towards the posterior lateral corner of the array.

Stimulus triggered z-scored response

In total, 96 electrodes were stimulated and EMG responses from 13 muscles were recorded and rectified. EMG responses from 20 ms before the stimulation to 40 ms after the stimulation were examined and averaged for all trials per electrode. The responses were then z-scored using the baseline in EMG activity from -20 ms before stimulation to -12.5 ms before stimulation. Valid muscle responses were greater than a z-score of 2 or less than a z-score of -2 for at least 2 consecutive milliseconds. The responses for two individual electrodes are shown in figure 3 and figure 4.

Figure 3 shows the responses elicited by stimulating an electrode in monkey H. For this particular electrode, the R sternohyoid and L genioglossus did not produce any valid responses. However, the L and R anterior digastrics, geniohyoids, mylohyoids, styloglossus, R genioglossus, and L sternohyoid were actively inhibited while the L superficial masseter was active. All muscles were actively inhibited except for the jaw depressor muscle. Similar figures were produced for all 96 electrodes however; the responses for just one electrode are depicted here.

Figure 4 shows the stimulus triggered average response for one electrode in monkey M. For this particular electrode, the jaw depressors including the R anterior digastric, and the L and R geniohyoid are active. The tongue protractor muscles, L and R genioglossus are also active. The L tongue retractor (L hyoglossus) is also active. Based on the EMG responses, this electrode represents jaw depression and asymmetrical tongue protrusion.

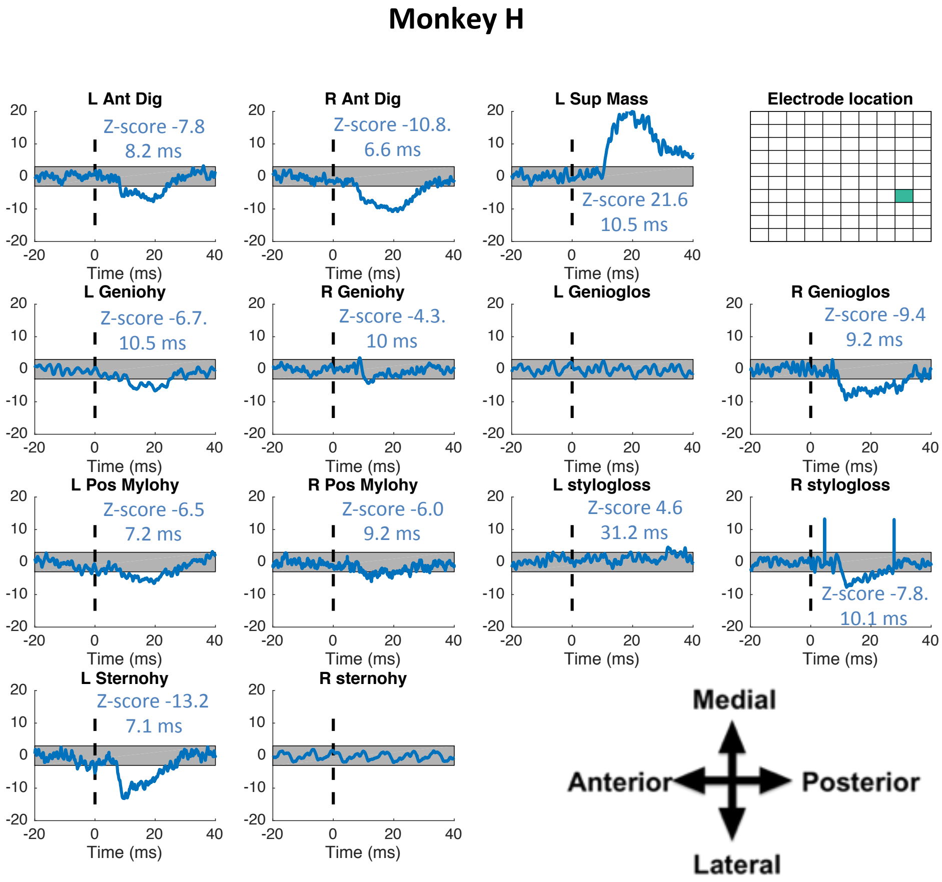


Figure 4.3. Stimulus triggered average EMG response for all 13 muscles at one electrode site in monkey H. Each subplot represents one muscle. The y-axis shows the z-scored response while the x-axis shows the time (ms). Negative times represent the number of milliseconds prior to stimulation while positive times represent the number of milliseconds after stimulation. The vertical dotted line at 0 ms represents the time of stimulation. The shaded gray box represents the arbitrary threshold used in this study. Muscle responses had to exceed either a z-score of 2 or -2 for 2 ms continuously. The figure in the top right corner depicts the location of the electrode within monkey M's array.

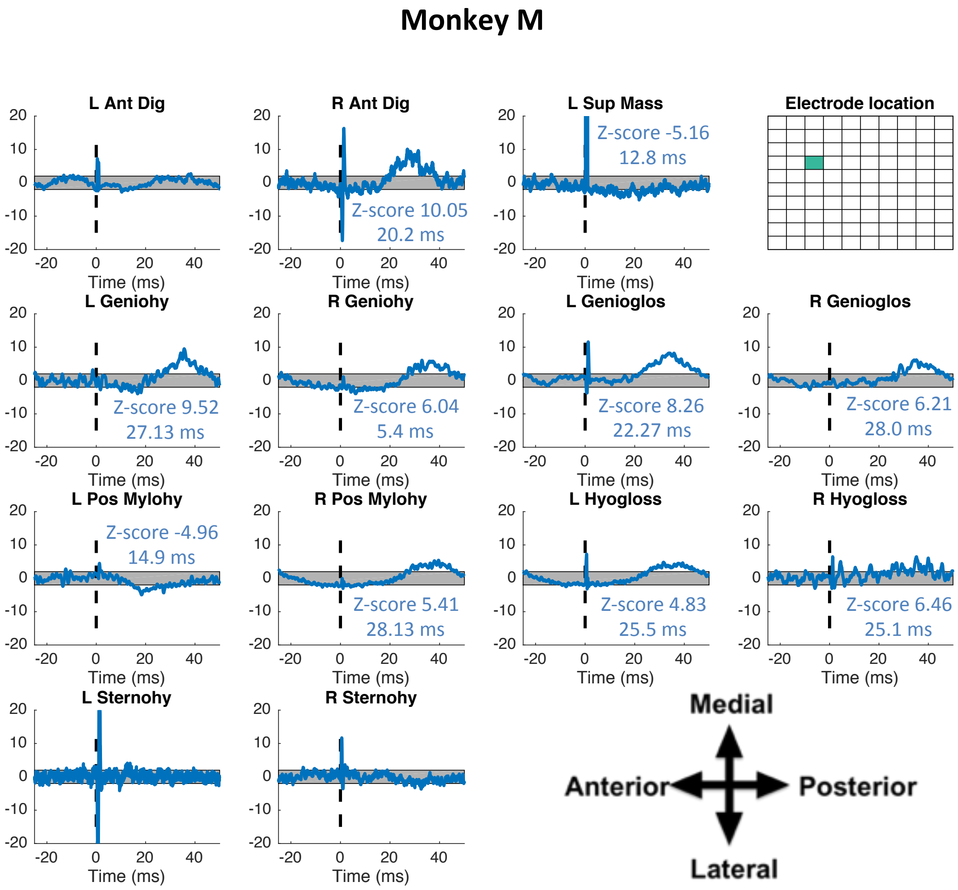


Figure 4.4 Stimulus triggered average EMG response for all 13 muscles at one electrode site in monkey M. Each subplot represents one muscle. The y-axis shows the z-scored response while the x-axis shows the time (ms). Negative times represent the number of milliseconds prior to stimulation while positive times represent the number of milliseconds after stimulation. The vertical dotted line at 0 ms represents the time of stimulation. The shaded gray box represents the arbitrary threshold used in this study. Muscle responses had to exceed either a z-score of 2 or -2 for 2 ms continuously. The figure in the top right corner depicts the location of the electrode within monkey M's array.

Response time

Table 4.2 Mean Response Time (ms)

Muscle	Innervation	Monkey H	Monkey M
L Superficial Masseter	V3	14.8 ± 7.0 ms	16.0 ± 9.6 ms
L Anterior Digastric		17.7 ± 9.3 ms	13.0 ± 10.3 ms
R Anterior Digastric		14.2 ± 7.4 ms	18.6 ± 10.4 ms
L Geniohyoid	C1-C3	13.6 ± 8.7 ms	12.3 ± 6.8 ms
R Geniohyoid		13.2 ± 7.7 ms	9.5 ± 8.79 ms
L Posterior Mylohyoid	V3	13.2 ± 7.8 ms	16.1 ± 7.0 ms
R Posterior Mylohyoid		14.4 ± 8.2 ms	21.5 ± 12.4 ms
L Sternohyoid	C1-C3	11.1 ± 6.2 ms	18.0 ± 14.1 ms
R Sternohyoid		18.1 ± 10.1 ms	10.5 ± 8.0 ms
L Styloglossus	XII	16.7 ± 8.9 ms	
R Styloglossus		15.7 ± 10.2 ms	
L Hyoglossus			12.3 ± 8.1 ms
R Hyoglossus			22.2 ± 9.2 ms
L Genioglossus		14.2 ± 8.8 ms	12.9 ± 11.4 ms
R Genioglossus		16.2 ± 10.0 ms	16.7 ± 8.1 ms

A muscle response had to exceed either a z-score of 2 or -2 for 2 ms consecutively. The first time point to meet these criteria represents response onset. The mean response onset time was calculated for the 13 muscles across all 96 electrodes (table 2). The response time for all muscles at each electrode is depicted in figure 5 for monkey H and figure 6 for monkey M. For monkey M, both anterior digastrics, both geniohyoids, R geniooglossus, R Mylohyoid, and L sternohyoid have the fastest response times in the medial portion of the electrode. For monkey M, R geniohyoid, L hyoglossus, and R sternohyoid have the fastest response time at the center of the array and the slowest response times in the anterior medial corner. The opposite trend is observed in the L superficial masseter and L posterior mylohyoid.

A Kolmogorov-Smirnov test was used to confirm that all mean onset times represented a normal distribution ($p \leq 0.05$) (as opposed to a uniform distribution from 0 ms to 40 ms). In monkey H, L sternohyoid had the shortest response time (11.1 ± 6.2 ms) while R sternohyoid had the largest response time (18.1 ± 10.1 ms). In monkey M, R geniohyoid had the shortest response time (9.5 ± 8.79 ms) while R hyoglossus had the longest response time (22.2 ± 9.2 ms). Both the sternohyoid and the geniohyoid are innervated by the anterior rami of C1 to C3 while the styloglossus is innervated by the hypoglossal nerve. In both individuals, the sternohyoid had the largest standard deviation in response times (R sternohyoid 10.1 ms in monkey H and L sternohyoid 14.1 ms in monkey M). However, L sternohyoid had the smallest standard deviation in response times in monkey H (6.2 ms), while another muscle innervated by anterior cervical rami, L geniohyoid had the lowest standard deviation in response times in monkey M. Thus, cranial nerves do not have less variable response times than anterior cervical rami.

Table 4.3 Mean Response Time by Innervation (ms)

Innervation	**Monkey H**		**Monkey M**	
	Excitatory	**Inhibitory**	**Excitatory**	**Inhibitory**
V3	11.91 ± 6.83	10.99 ± 8.79	12.20 ± 9.01 *	12.28 ± 9.10 *
XII	15.92 ± 7.38	11.62 ± 8.27	16.70 ± 9.48	14.48 ± 8.34 *
C1-C3	13.29 ± 8.34	9.78 ± 7.12	15.36 ± 9.84	17.17 ± 11.08

* Value is significantly less than that of C1- C3 muscles based on one-tailed two-way t-test with unequal variance

The mean and standard deviation in response times for all muscles innervated by particular nerves were calculated for excitatory and inhibitory responses. For both monkeys, the mandibular nerve V3 had the fastest response times for excitatory responses. However, the difference between V3 and C1-C3 muscle response times was significant only in monkey M (excitatory $p = 0.027$, inhibitory $p < 0.001$). In monkey M, the hypoglossal nerve (XII) also had a significantly lower inhibitory response time compared to C1-C3 muscles ($p = 0.026$). In monkey H, C1-C3 had lower excitatory and inhibitory response times compared to the hypoglossal nerve (XII). In fact, C1-C3 muscles had the fastest inhibitory response times in monkey H. The inhibitory response times for hypoglossal muscles was significantly greater than the inhibitory response times for muscles innervated by C1-C3 ($p = 0.040$).

Table 4.4 Number of Responses

Muscle	Monkey H		Monkey M	
	Ipsilateral	Contralateral	Ipsilateral	Contralateral
Anterior Digastric	40	58*	83*	21
Geniohyoid	66	54	79*	56
Posterior Mylohyoid	61*	36	63*	37
Sternohyoid	31	21	12	50*
Styloglossus	29	46*		
Hyoglossus			50*	5
Genioglossus	25	56*	50	70*
TOTAL	252	271	337*	239

* Value is significantly greater than 0.5 probability based on a one-tailed binomial probability distribution test

Number of responses

The number of electrodes that elicited a response varied between muscles and between individuals. Both monkeys had arrays implanted in the left hemisphere of MIo. In monkey H, L genioglossus elicited responses from just 25 out of 96 electrodes while R genioglossus elicited responses from 56 out of 96 electrodes. Meanwhile, monkey M's L hyoglossus elicited responses from 50 electrodes out of 96 while R hyoglossus elicited responses from just 5 out of 96 muscles. In some muscles, the number of responses between ipsilateral and contralateral sides varied greatly. However, in other muscles, this difference was less pronounced. For example, in monkey H, the contralateral geniohyoid produced responses in 54 electrodes while the ipsilateral geniohyoid evoked responses from 66 electrodes out of 96. Interestingly, the ipsilateral posterior mylohyoid produced significantly more responses than the contralateral muscle in both monkeys ($p = 0.007$ in monkey H, $p = 0.006$ in monkey M). In both monkeys the contralateral genioglossus also produced more responses than the ipsilateral muscle ($p < 0.001$ in monkey H, $p = 0.04$ in monkey M). In monkey H, contralateral muscles did respond more frequently than ipsilateral muscles, however this difference was not significant. In monkey M, the ipsilateral muscles produced significantly more responses than contralateral ones ($p < 0.001$).

Response Strength

Figures 7 and 8 show the extrema responses for each electrode. Each subplot represents one muscle and the coloring represents the strength of the response. For monkey H, the anterior medial corner of the array shows an inhibitory response toward jaw depressor muscles and an excitatory response towards the jaw elevator muscle (L superficial masseter). The inverse is true in monkey M. In monkey M, jaw depressors like R anterior digastric and R geniohyoid are excited while the jaw elevator is clearly inhibited.

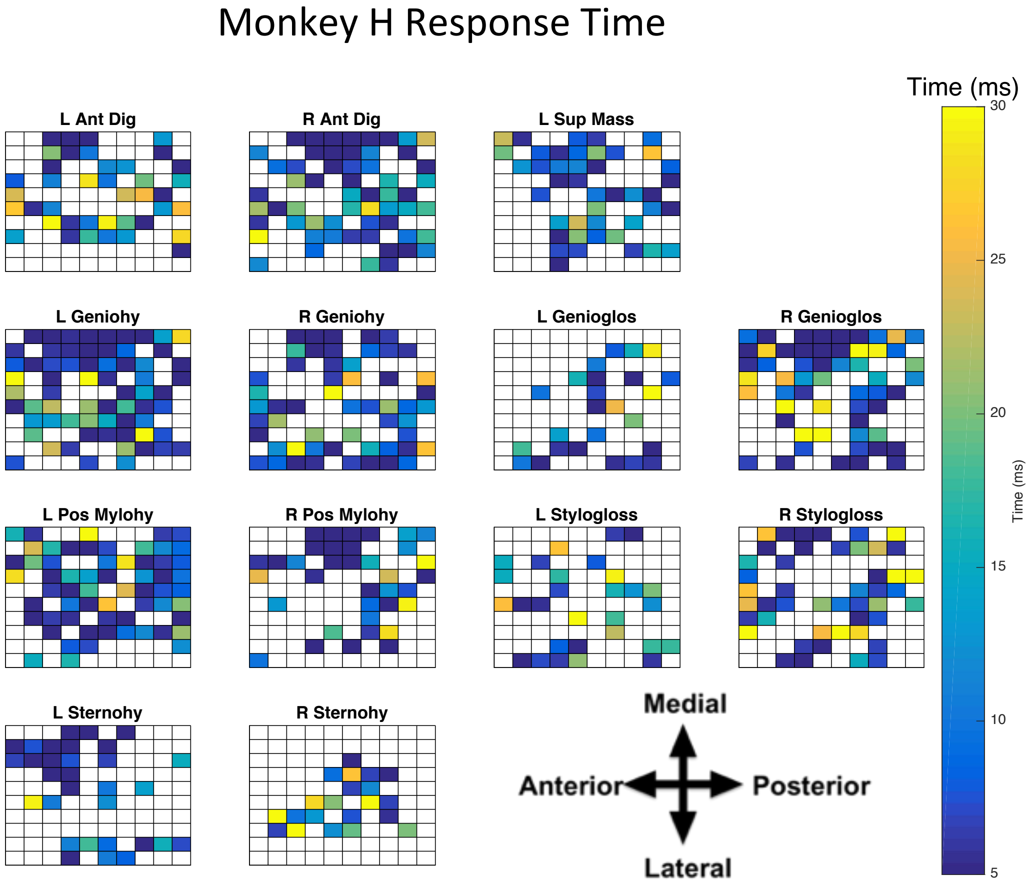


Figure 4.5 Shows the response time for each electrode in milliseconds for monkey H. Each subplot represents one muscle. The response onset times are illustrated as colors and depicted in the color bar to the right of the figures.

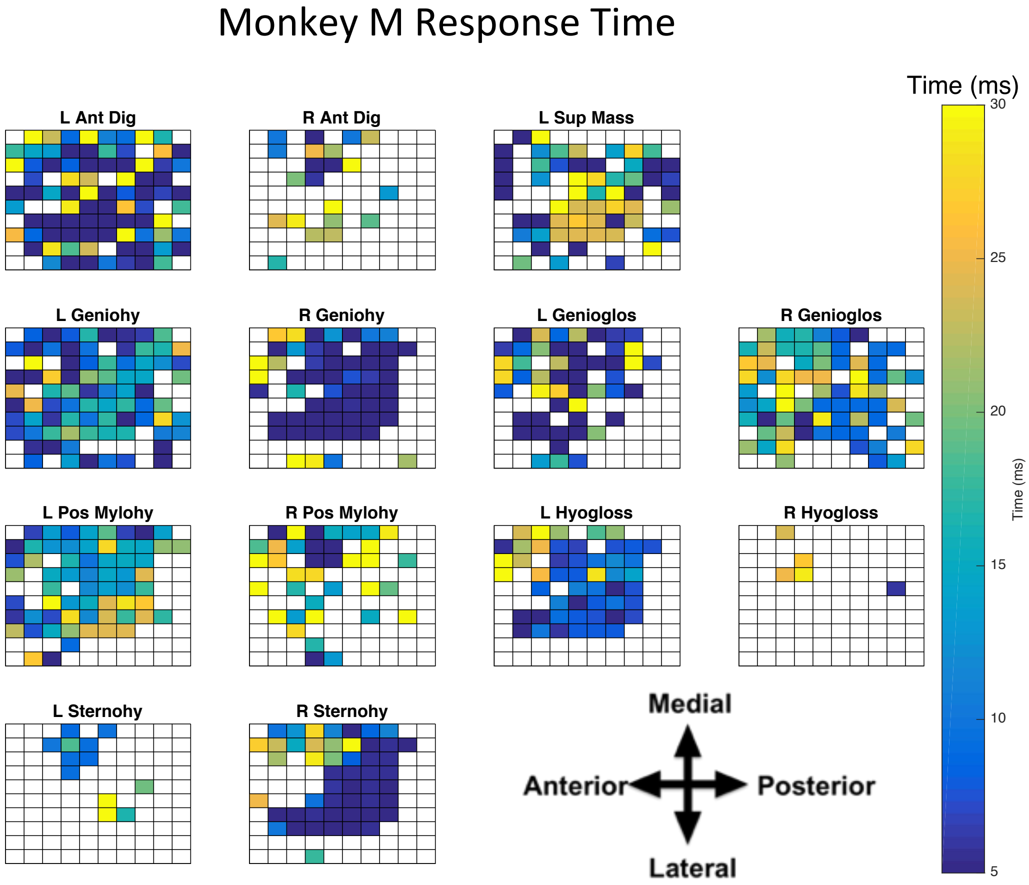


Figure 4.6 Shows the response time for each electrode in milliseconds for monkey M. Each subplot represents one muscle. The response onset times are illustrated as colors and depicted in the color bar to the right of the figures.

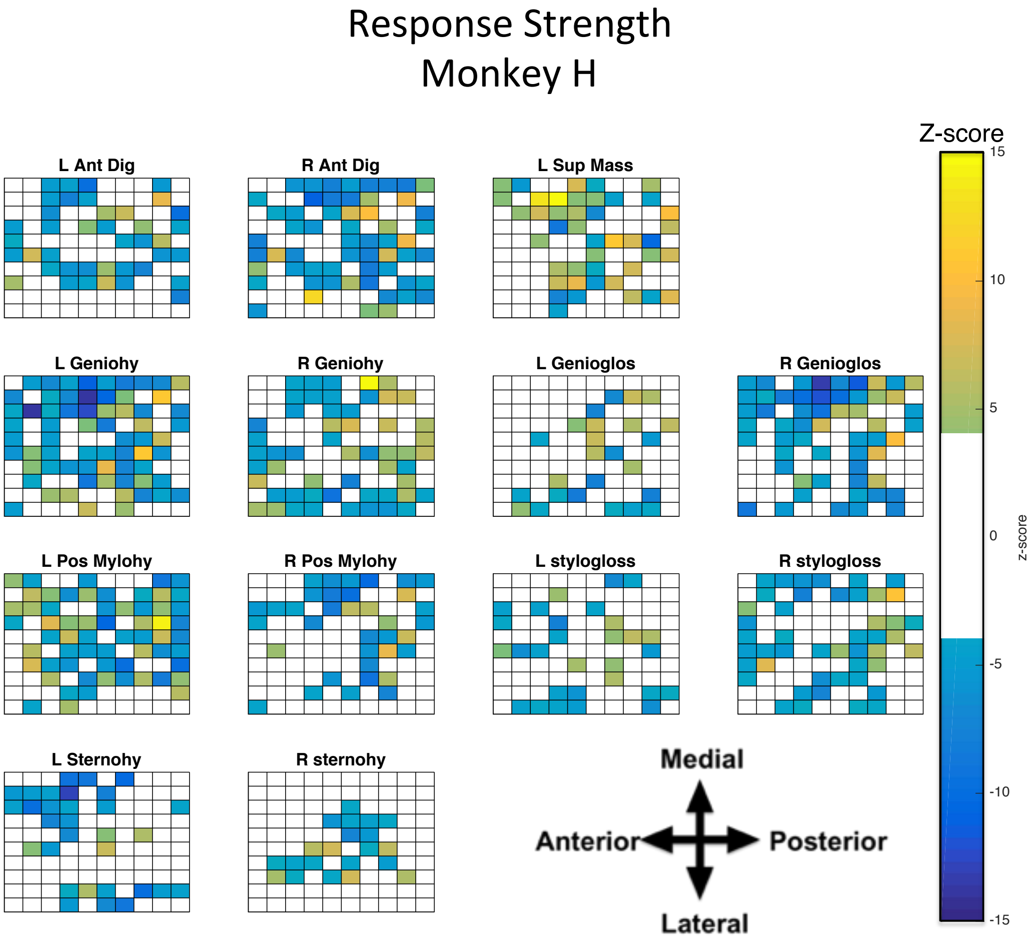


Figure 4.7 The extrema of the z-score muscle responses for each electrode in Monkey H are plotted as a color. Each subplot represents one muscle and the color-coding is depicted in the color bar to the right. Electrodes that did not produce a response are shown in white.

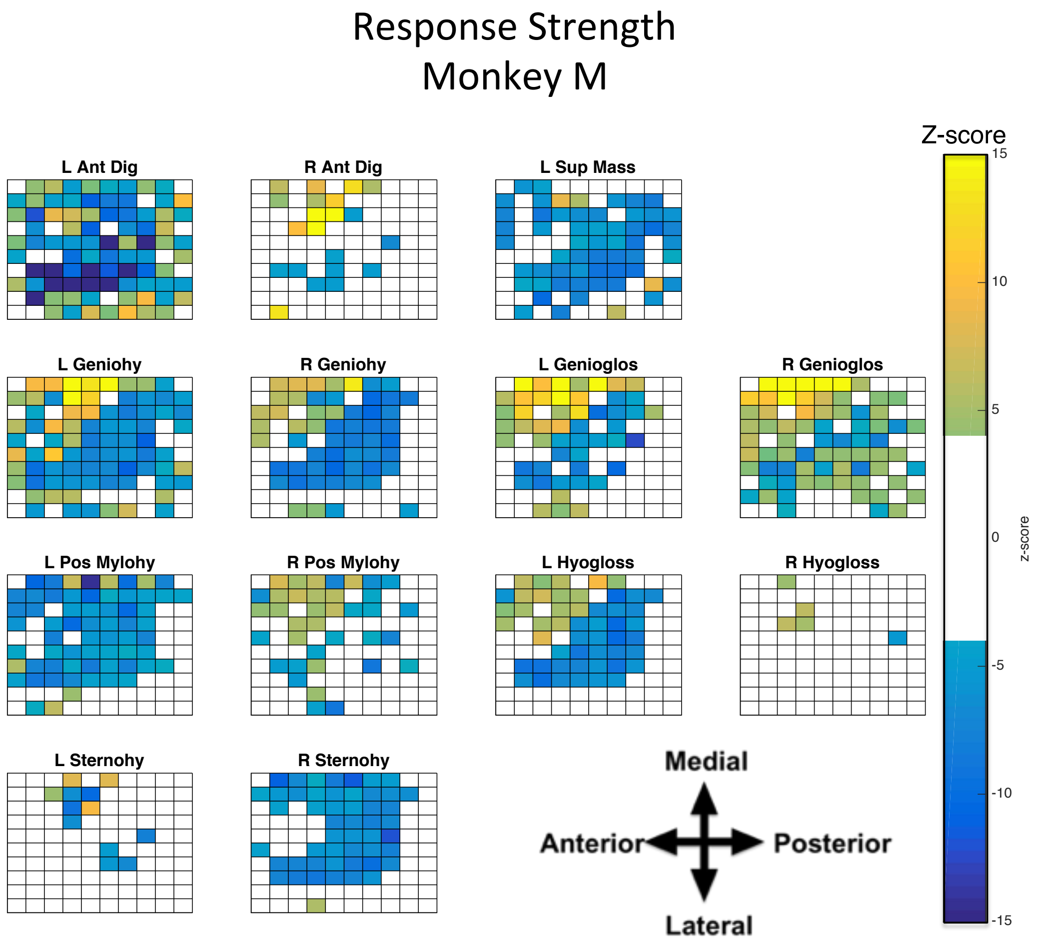


Figure 4.8 The extrema of the z-score muscle responses for each electrode in Monkey M are plotted as a color. Each subplot represents one muscle and the color-coding is depicted in the color bar to the right. Electrodes that did not produce a response are shown in white.

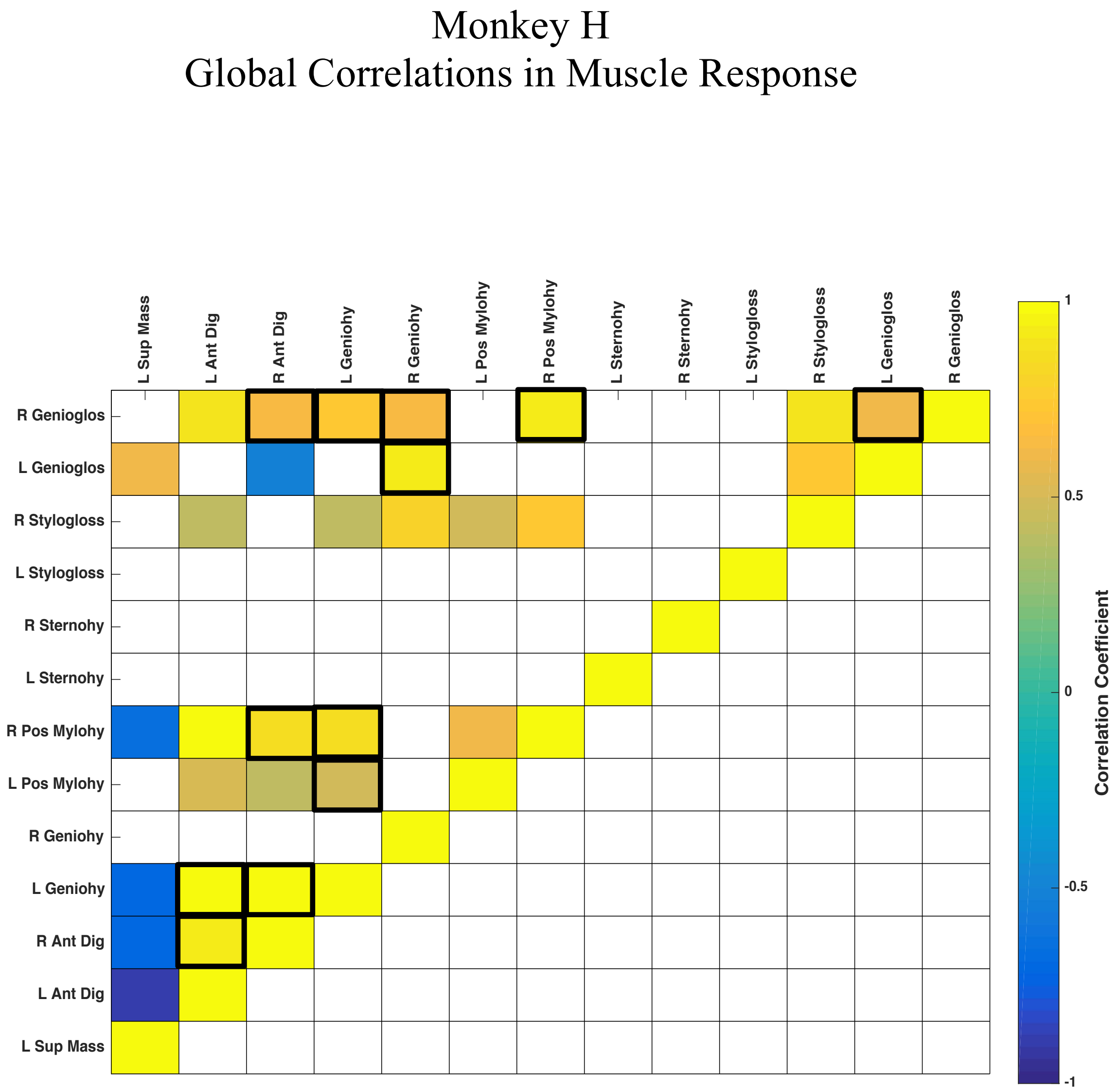


Figure 4.9. Cross correlations in muscle responses for monkey H. The strength of the correlation is shown as a color and responses common to both monkeys are shown within a black box.

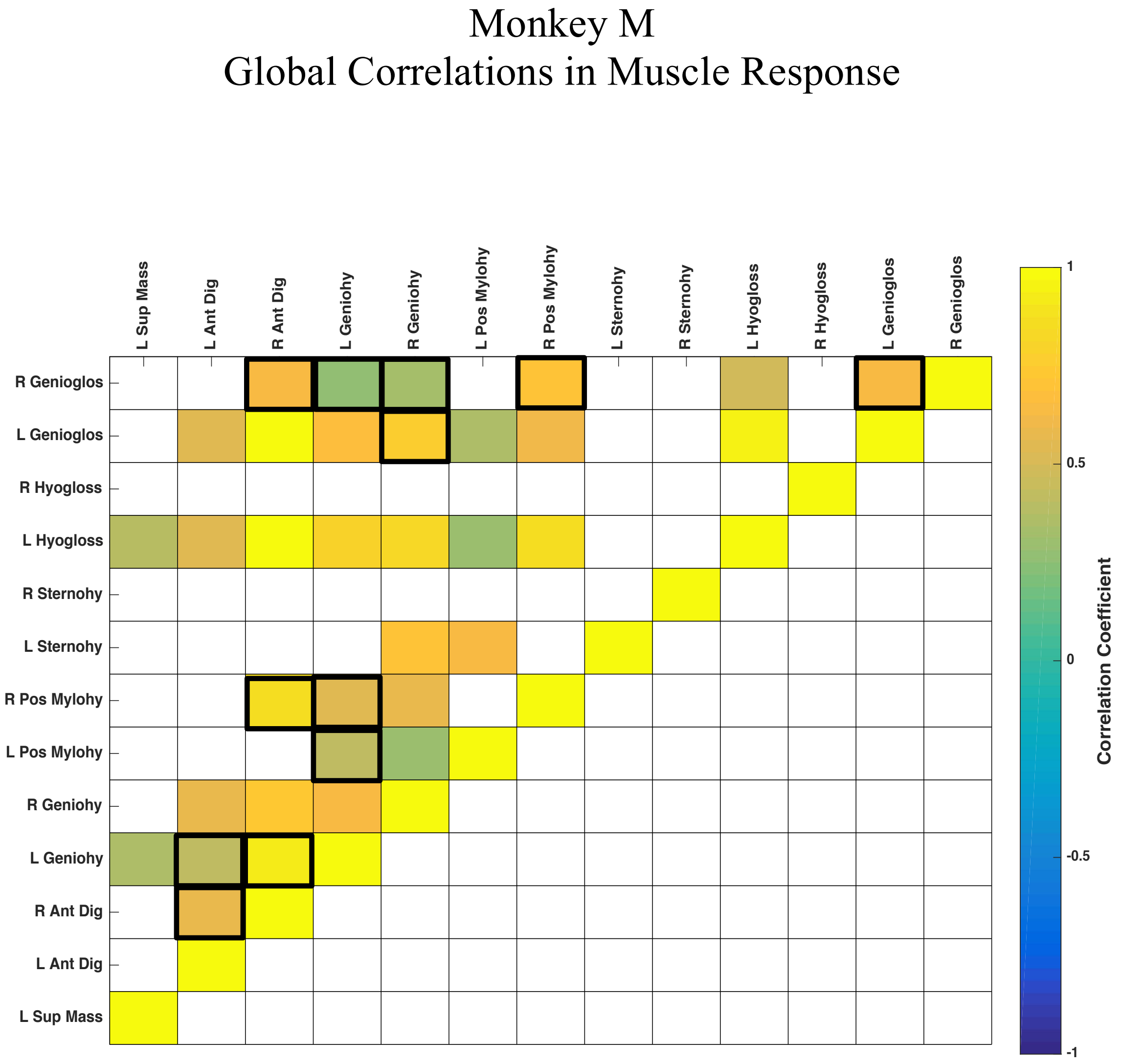


Figure 4.10. Cross correlations in muscle responses for monkey M. The strength of the correlation is shown as a color and responses common to both monkeys are shown within a black box.

Global Correlations

Cross correlations in muscle response (+1 for activation and -1 for inhibition) with 0 ms lag time were used to identify global correlations between muscles. Significant correlations between muscle pairs are shown in figures 9 and 10 ($p < 0.05$). Correlations that are common to both monkeys are shown in black boxes. Both excitatory and inhibitory reactions were observed in monkey H but only excitatory reactions were observed in monkey M. The single jaw elevator muscle included in monkey H's EMG recordings had a strong inhibitory correlation with jaw depressors including both anterior digastrics and the ipsilateral geniohyoid. However, it had a positive correlation with the ipsilateral tongue protruder. Additionally in monkey H, the contralateral tongue retractor was positively correlated with three out of four jaw depressors and five out of six hyoid elevators. The ipsilateral mylohyoid, which depresses the hyoid and elevates the floor of the mouth, was positively correlated with both jaw depressors indicating that jaw depression is often accompanied by mylohyoid activity.

In both monkeys, positive correlations were observed between the jaw depressor and hyoid elevator muscles including the two anterior digastric muscles, ipsilateral geniohyoid to both anterior digastrics, the mylohyoid to the contralateral geniohyoid and ipsilateral anterior digastric. These findings suggest that jaw depression and hyoid elevation is often accompanied by elevation of the floor of the mouth. Additionally, both genioglossus muscles are positively correlated to jaw depressors and hyoid elevators including contralateral and ipsilateral geniohyoids, R genioglossus to R anterior digastric, and R genioglossus to R mylohyoid. The L and R genioglossus muscles are also positively coordinated. Thus, tongue protrusion is often accompanied by jaw depression and hyoid elevation. However in monkey H R anterior digastric is negatively correlated to L genioglossus.

In addition to the correlations common to both monkeys, monkey M also shows a positive correlation between both L genioglossus and L hyoglossus with all four jaw depressor muscles. These findings suggest that both tongue protrusion and retraction are closely associated with jaw depression and that although tongue protruders and retractors are antagonistic, they are often activated and inhibited together. Neither monkey showed any significant correlations for L and R sternohyoids. These findings indicate that the sternohyoid is not consistently used to fix the hyoid while the hyoid elevators/ jaw depressors are active.

K-means clustering

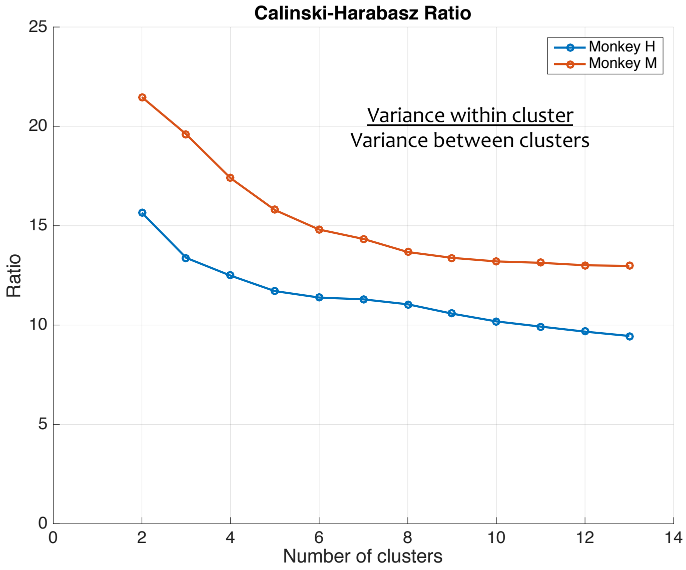


Figure 4.11. The Kalinski-Harabsz criterion was used to identify the ideal number of clusters in monkey H (blue) and Monkey Y (red). This figure shows the number of clusters on the x-axis along with the within cluster variation to between cluster variation ratio on the y-axis.

The Calinski-Harabasz criterion identified two discrete clusters of muscle response strengths in monkey H. K-means clustering was used to sort the muscle responses for each electrode in monkey H's MIo array into these two clusters. Cluster one was located anterolaterally while cluster two was located anteromedially in the array. The posterior portion of the array was a mix of the two clusters. The mean z-score response was calculated for each cluster and only responses that were significantly different from a mean z-score of zero based on a one-way t-test were pictured in figures 13 and 14. Figure 13 shows the mean z-score muscle responses for all electrodes in cluster one. The ipsilateral jaw elevator, hyoid depressor, and tongue retractor are all actively inhibited while both anterior digastric muscles and the ipsilateral geniohyoid are actively excited. Thus, this cluster represents jaw depression. In cluster two both anterior digastric muscles, the ipsilateral geniohyoid, mylohyoid, the ipsilateral mylohyoid, contralateral styloglossus, and contralateral genioglossus are all actively inhibited. The only muscle that is actively excited is the only jaw elevator, the superficial masseter. Cluster two may represent jaw elevation and active inhibition of the tongue muscles. The locations of monkey H's two clusters confirm the findings of Huang et al. (1988). The cluster representing jaw elevation is located more medially while the cluster representing jaw depression, hyoid elevation, and inhibition of the tongue retractor is located more laterally. Moreover, this gradient is oriented in a diagonal manner and starts in the anteromedial corner of the array, similar to the findings of Huang et al. (1988). In both monkeys, muscles that are not relevant to the specific action produced by the cluster are actively inhibited.

The optimal number of clusters in monkey M was also two. Cluster one was located centrally in the array, while cluster two was concentrated in the anteromeadial and posterolateral corners of the array. In cluster one, all muscles were actively inhibited. In cluster two the ipsilateral superficial masseter, mylohyoid, and contralateral sternohyoid are inhibited. The contralateral anterior digastric muscle and both geniohyoids are active indicating jaw depression. Both genioglossus muscles are also active indicating tongue protrusion. However, the ipsilateral hyoglossus is also active. The hyoglossus muscle retracts the tongue. This cluster appears to represents jaw depression and hyoid elevation and asymmetric tongue protrusion. However, because the mylohyoid is actively inhibited, the hyoid is not related to swallowing.

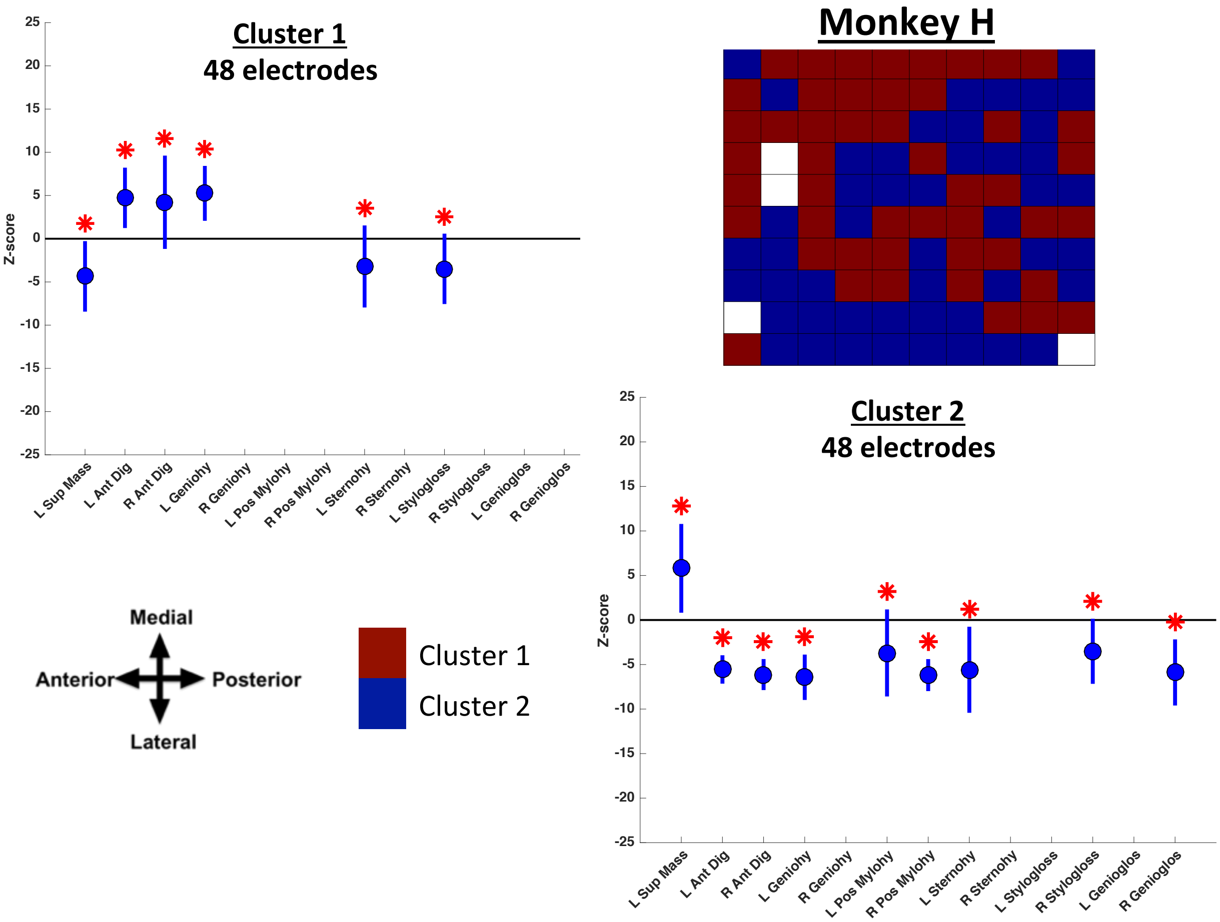


Figure 4.12. Each of the electrodes in monkey H's array were sorted into clusters. The figure to the top right shows the spatial locations of the electrodes within each cluster. The mean z-scored response strength for each muscle was also calculated for both clusters. The mean z-score is shown on the y-axis and each of the muscles are shown on the x-axis. The mean value is shown as a dot, the standard deviation is shown as a blue line and significant responses have a red asterisk above them.

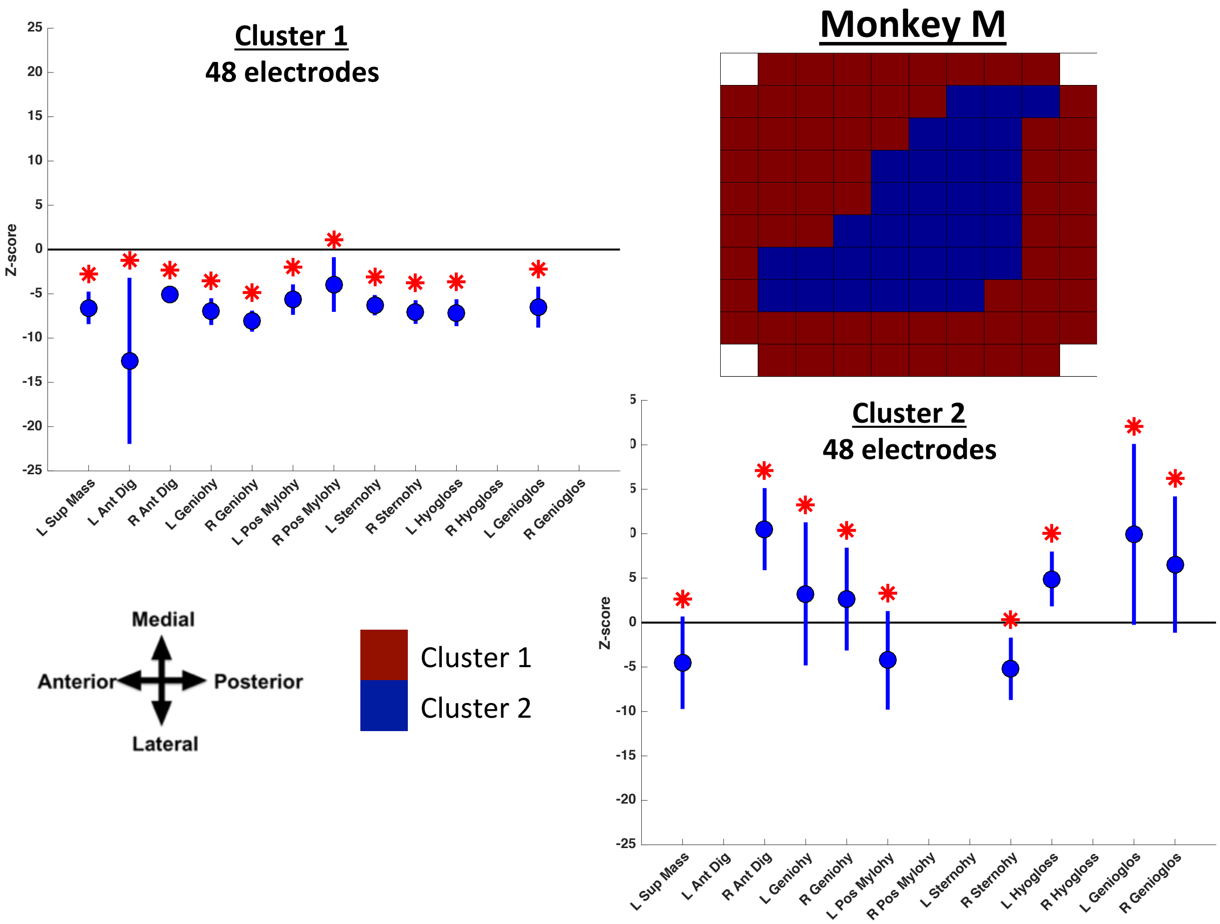
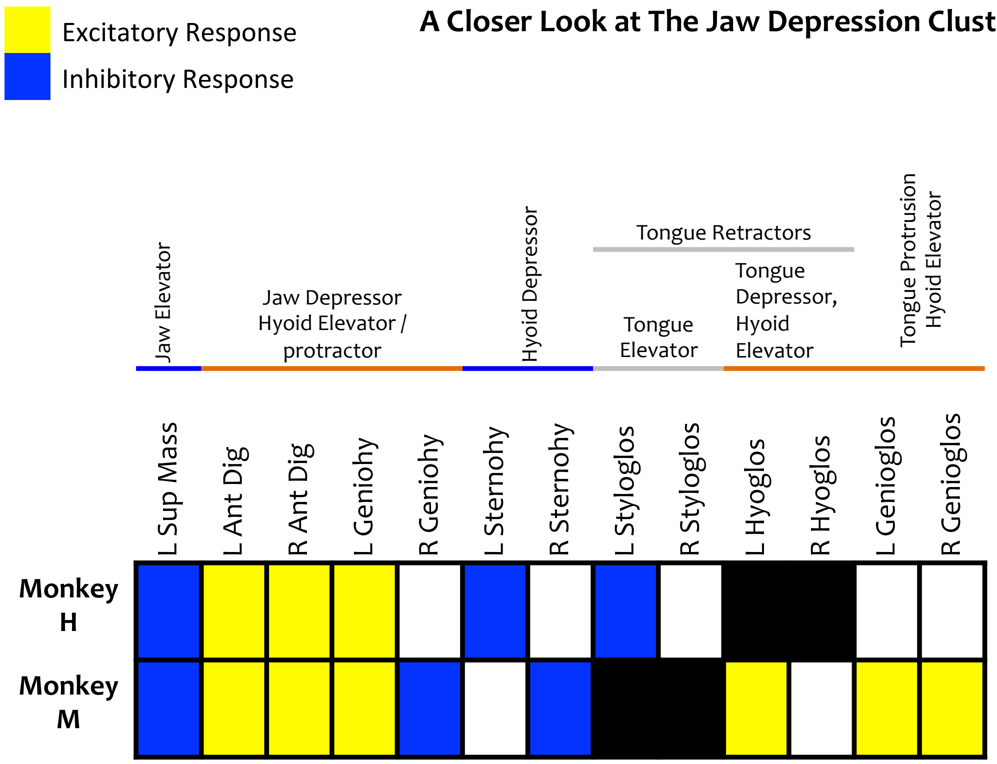


Figure 4.13. Each of the electrodes in monkey M's array were sorted into clusters. The figure to the top right shows the spatial locations of the electrodes within each cluster. The mean z-scored response strength for each muscle was also calculated for both clusters. The mean z-score is shown on the y-axis and each of the muscles are shown on the x-axis. The mean value is shown as a dot, the standard deviation is shown as a blue line and significant responses have a red asterisk above them.



🟨 Excitatory Response

🟦 Inhibitory Response

A Closer Look at The Jaw Depression Cluster

	Jaw Elevator	Jaw Depressor Hyoid Elevator / protractor				Hyoid Depressor		Tongue Retractors				Tongue Protrusion Hyoid Elevator	
								Tongue Elevator		Tongue Depressor, Hyoid Elevator			
	L Sup Mass	L Ant Dig	R Ant Dig	L Geniohy	R Geniohy	L Sternohy	R Sternohy	L Styloglos	R Styloglos	L Hyoglos	R Hyoglos	L Genioglos	R Genioglos
Monkey H	🟦	🟨	🟨	🟨	⬜	🟦	⬜	🟦	⬜	⬛	⬛	⬜	⬜
Monkey M	🟦	🟨	🟨	🟨	🟦	⬜	🟦	⬛	⬛	🟨	⬜	🟨	🟨

Figure 4.14. Jaw depression cluster in both monkeys. Excitatory responses are shown in yellow and inhibitory responses are shown in blue. Muscles without any data are shown in black. No significant response is shown in white. The function of each set of muscles is listed above the table.

Jaw depression cluster

Both monkeys had a cluster that represented jaw depression and hyoid elevation/ protraction. A comparison between these two clusters is presented in figure 4.14. In both monkeys, the jaw elevator, L superficial masseter is inhibited while the jaw elevators and hyoid elevators/ protractors the anterior digastric and L geniohyoid are active. In monkey H the L sternohyoid is inhibited while the R sternohyoid is inhibited in monkey M. The sternohyoid depresses the mandible. In monkey H, the tongue retractor L styloglossus was inhibited. However, in monkey M, the retractor muscle, L hyoglossus was active. This muscle has the additional effect of elevating the hyoid. Hence, in both monkeys, this cluster represents jaw depression and hyoid elevation/ protraction. However, in monkey M bilateral tongue protruders L and R genioglossus were also active while these muscles had no significant response in monkey H.

DISCUSSION

The purpose of this study is to determine whether behavior is arranged ethologically in MIo using the single biphasic pulse ICMS protocol developed by Park et al. (2001; 2004). The stimulus triggered mean EMG response was calculated for 13 orofacial muscles across at least 1500 stimulations. This protocol increases the precision and specificity of ICMS by minimizing current spread, and neural facilitation caused by increased amplitudes or repetitive stimulation. The findings of this study suggest that MIo is arranged into discrete microzones that each represent an ethologically relevant set of actions. Muscles that are not relevant to the specific action are actively inhibited. These microzones may potentially be modified in a use dependent manner. These findings are specifically relevant and interesting in the context of the orofacial system because MIo contains monosynaptic bilateral connections to motor neuron pools in the trigeminal, facial and hypoglossal nucleus. These bilateral connections facilitate coordination between the ipsilateral and contralateral orofacial muscles during specialized behaviors including feeding.

Response time

We predicted that muscles innervated by cranial nerves would have a faster response time and that excitatory responses would occur more rapidly than inhibitory responses. In both monkeys, the mandibular nerve (V3) had the fasted excitatory response times. However, this response time was significantly lower than muscles innervated by anterior cervical rami only in monkey M. In monkey H, muscles innervated by C1-C3 had faster excitatory and inhibitory response times than muscles innervated by the hypoglossal (XII) nerve. Further studies are needed to determine whether cranial nerves have a faster response time than anterior rami. Excitatory responses were not faster than inhibitory responses in both monkeys.

Bilateral responses

Previous anatomical studies have shown that MIo has strong bilateral connections to the facial, hypoglossal, and trigeminal motor nuclei (Kuypers 1958a; Sokoloff and Deacon 1992; Morecraft et al. 2001 2014). Morecraft et al. (2014) observed slightly predominant connections to the contralateral hypoglossal nucleus in four out of five *Macaca mulatta* studied. They noted that contralateral predominance in anatomical connectivity is accompanied by lower motor thresholds for evoked movement during ICMS and larger mean evoked motor potentials (Morecraft et al. 2014). However, Morecraft et al. (2014) also recognized that these findings were influenced by hemispheric dominance, handedness, and direction of naturally occurring tongue deviation that is present in all individuals (Morecraft et al. 2014).

The present study looked at both the strength and the number of responses evoked in ipsilateral versus contralateral muscles and found significantly more ipsilateral responses in one of the two monkeys. The other monkey did have more contralateral responses than ipsilateral responses, however this difference was not significant. It is possible that the two monkeys had different side preferences. Because the purpose of this study was not to evoke observable movement, it was not feasible to determine whether the threshold for movement was lower for contralateral muscles compared to ipsilateral muscles. It is important to note that neither the natural tongue deviation nor the handedness was recorded for both monkeys. However, it is possible to determine whether side preference is directly correlated to an increased number of monosynaptic cortico-motoneuronal connections between the ipsilateral and contralateral muscles in these specific monkeys via post mortem anatomical studies.

Global correlations

We predicted that muscles with synergistic actions would be positively correlated with each other while antagonistic muscles would be negatively correlated with each other. The present study does support the hypothesis that synergistic muscles are positively correlated. In both monkeys, jaw depression, tongue protrusion, and hyoid depression were all positively correlated with each other. However, tongue retraction was also positively correlated jaw depression and hyoid elevation. In both monkeys, the bilateral tongue retractors were not positively correlated to each other, indicating that these muscles are often used asymmetrically and in association with tongue protruders to twist the tongue or move it laterally. Thus, antagonistic muscles are not always negatively correlated to each other. In both monkeys, the sternohyoid was not positively coordinated with any other muscles suggesting that the sternohyoid may not be utilized in these monkeys to fix the hyoid in place during jaw depression.

Ethological clustering

Huang et al. (1988) recognized that 53% of the loci they stimulated produced “mixed responses” because they included responses from the face, jaw, and tongue muscles. The present study confirms that jaw representation is located more medially than hyoid and tongue representation. Moreover, we confirm that the jaw is represented in a diagonal band that is oriented anteromedially to posterolaterally. Huang et al. also recognized that MIo consists of a mosaic of multiple and discrete microzones representing specific movements (1988). The present study identified these discrete microzones within the MIo of both monkeys. The microzones represented ethologically relevant behaviors. In both monkeys, one cluster represented jaw depression, hyoid elevation/ protraction.

There were two distinct functional clusters of muscle representation in monkey H. The first cluster represented jaw depression, and active inhibition of the jaw elevator, tongue, and hyoid muscles. This cluster was located in the anterior lateral portion of the array. The second cluster represented jaw elevation and active inhibition of the jaw depression, hyoid, and tongue muscles. It was located in the anterior medial portion of the array. The intermediate area between these two clusters was occupied by a combination of jaw elevation and jaw depression.

Monkey M also had two functional clusters. The first cluster represented active inhibition of all muscles except the contralateral tongue retractor and ipsilateral tongue protruder. The second cluster represented jaw depression and asymmetric tongue protrusion. In both monkeys, behaviors were arranged diagonally on the array as oppose to medial to lateral.

A number of studies have reported that the proportion of cortical sites that represent jaw depression is much larger than the number of sites that represent jaw elevation (Clark and Luschei 1974; McGuinness et al. 1980; Gould et al. 1986; Huang et al. 1988). In monkey M, we did not find any electrodes that evoked jaw elevation. However, in monkey H, 47 sites evoked jaw elevation while 49 sites evoked jaw depression. Incidentally, both monkeys were specifically trained on a precision bite task for two to three hours a day, five days a week prior to this study. However, monkey H spent four months on this task while monkey M spent two months on the task. These findings may support the hypothesis that MI is a dynamic construct consisting of multiple efferent microzones and that each microzone represents an ethologically relevant action. However, it is also possible that differences in the clusters observed between the two monkeys are due to minute differences in the placement of the two arrays.

Past studies have shown that the neurophysiological correlates of behavior present within MIo can be modified in a use dependent manner (Murray and Sessle 1992; Schieber 2001; Sessle et al. 2005). However, further studies are needed to determine whether the increased representation of jaw elevation in monkey H was due to persistent training on a precision bite task. One way to test whether the action clusters within MIo change based on individual experience is to repeat this experiment before and after training primates on tasks that target specific muscle groups like precision biting or force production by the tongue. If the functional groups of muscles represented in the cortex are modified based task specific training, we would expect to see increased representation of the specific muscles involved in the trained task and potentially increased inhibition of irrelevant muscles.

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DISCUSSION

Many researchers have posited that mammalian motor patterns are conserved during mastication (Hiiemae 1978; Bramble and Wake 1985; Weijs 1994). While Weijs (1994) was not the first researcher to recognize that transverse jaw movement during jaw closing is produced by asymmetric activation of the superficial masseter, medial pterygoid, and posterior temporalis (Herring 1973 1976 1979; Gorniak 1977 1985; Weijs and Dantuma 1980), he did reify the triplet motor pattern into an ancestral motor pattern modified by natural selection to produce the range of motor patterns observed in extant mammals. However, there is little evidence to suggest that masticatory motor patterns are homologous.

“Homology is the relationship of two characters that have descended, usually with divergence, from a common ancestral character” (Fitch 2000, 1). Presently, there is no direct evidence that motor patterns are a phenotype that demonstrates evolutionary and developmental autonomy. Rhythmic behaviors composed of specific motor patterns are affected by regulating genes that influence CPG neurons in the brainstem (Viemari et al. 2004). Specific genes that affect the masticatory motor pattern have yet to be identified. There is insufficient direct evidence to corroborate or falsify the hypothesis that mammalian masticatory motor patterns are homologous.

Instead, Hylander and colleagues relate variation in the existence of the triplet motor pattern to variation in jaw morphology and kinematics (Hylander and Johnson 1985 1994; Hylander et al. 1987 2000 2004 2005). Support for the triplet hypothesis is variable and clade specific.

Marsupial and ungulate jaw elevator motor patterns provide little support for Weijs' model of triplet motor pattern evolution: a triplet motor pattern is only observed in one marsupial and (partially) one ungulate (Crompton et al. 2008a; Crompton et al. 2008b; Vinyard et al. 2005; Williams et al. 2007). In primates the triplet motor pattern has been identified in *Sapajus* and *Cebus* (Williams et al. 2011), *Homo* (Moller 1966; Langenbach and Hannam 1999), *Papio*, and *Macaca*, but not in *Aotus* and *Callithrix* (Hylander and Johnson 1994; Hylander et al. 2000 2005; Vinyard et al. 2006). Nonetheless, among biomechanists, the presence or absence of the triplet motor pattern has largely been treated as a binomial trait (Williams et al. 2011).

Computational studies of a variety of tasks suggest that movement primitives (simple spatiotemporal elements or building blocks) of behaviors, such as mastication, may represent local optima emerging from a learning process, whereby functional criteria are applied to a range of possible ways of moving until a local functional optimum is reached (Flash and Hogan 1985). Neuroscientists have postulated that the CNS generates behavior by activating these modules of muscle recruitment often referred to as muscle synergies (Bizzi et al. 1991; Mussa-Ivaldi et al. 1994; Alessandro et al. 2013). Muscle synergies are invariant and hard-wired patterns of activation (timing and amplitude) across specific muscles (Overduin et al. 2008). From the neural and motor control perspectives, muscle synergies can serve one of two functions: They may be a constraint on movement control; or they may be an optimization strategy (Nazarpour et al. 2012). Other workers in the field of neuroscience have advocated the uncontrolled manifold hypothesis, which suggests that the CNS identifies and optimizes only task relevant variables. Variability that does not affect the behavioral goal is allowed since correcting task irrelevant variability is an unnecessary waste of effort (Tresch and Jarc 2009). The muscle synergy hypothesis and the uncontrolled manifold are not mutually exclusive.

The overarching goal of my dissertation was to unite these two disparate fields and enable increased information transfer. Specific aim I determined the extent to which the triplet motor pattern is conserved during mastication. Specific aim II determined whether the CNS views the triplet motor pattern as a unit of control. It suggests concise and quantifiable definitions for unison, synchrony, and coordination to facilitate the flow of information between the fields of neuroscience, biomechanics, and dynamical systems. Specific aim III determines how functional groups of muscles are organized within the cortex.

AIM I

Aim I sought to identify the proportion of cycles that follow the triplet motor pattern in five different species of primates including, *Eulemur fulvus*, *Propithecus verreauxi*, *Papio anubis*, *Macaca fascicularis*, and *Pan troglodytes*. The findings of this study are that the *mean* peak activity times for the jaw elevator muscles follow the triplet motor pattern in all five species studied. Moreover, the *prevalence* of the triplet motor pattern is also greatest at peak muscle activation compared to onset and offset. However, there was variability in the prevalence of the triplet motor pattern between species (ranging from 20% in *Pan* to 81% in *Propithecus* at peak muscle activation). These findings suggest that the triplet motor pattern may be functionally relevant at specific points in the masticatory cycle. Flexibility in muscle activation patterns indicates that the jaw elevator motor pattern is not a fixed binomial trait. Instead we hypothesize that the triplet motor pattern may act as an “attractor” to the masticatory system, a state toward which a system progresses regardless of the initial state (American Heritage Dictionary of the

English Language 2000). This hypothesis suggests that if all external forces and factors acting on the system were constant, the triplet motor pattern would be one among a discrete and finite set of motor pattern observed. However, modifications to the motor pattern may be necessary to maintain kinematics given changing conditions, such as food bolus, condition and position. The triplet motor pattern may be one of multiple motor patterns that produce the kinematics and force production, required for successful mastication. Thus the a logical continuation of this study would be to determine the extent to which force production and kinematics are conserved within the specific masticatory cycles studied.

AIM II

Aim II sought to determine whether the triplet muscles form a unit of control for the CNS in the same five species mentioned in Aim I. Moreover, Aim II also sought to standardize quantifiable definitions for unison, synchrony, and coordination in order to facilitate information flow between the fields of biomechanics, neuroscience, and dynamic systems. This was the first study to analyze mastication in the phase domain. The findings of this study demonstrate that the triplets are not characterized by stereotyped and hard-wired patterns of activation. They are not *invariant* units of control for the CNS (Overduin et al. 2008). Triplet muscles are more highly coordinated during triplet cycles and non-triplet muscles are more tightly coordinated during non-triplet cycles, suggesting that different patterns of coordination predominate in different cycles. Instead, triplets appear to be flexibly recruited in some cycles and not others. Triplet II, which is recruited later in the cycle, during the slow close phase, is more variable than triplet I, which is recruited earlier in the cycle, during the fast close phase (Ross and Iriarte-Diaz 2014).

The findings of Aim II suggest that intrinsic criteria are used by the system to assess which motor pattern to recruit prior to cycle start. However, the recruited motor pattern is flexible and may be modified based on sensory feedback. This study supports the hypothesis that movements are composed of specific modules of muscle activation (amplitude and timing). However, these modules are modified to produce desired force production and kinematics. Thus the system may be controlling for task relevant variables while allowing variation in irrelevant dimensions. This study is a proof of concept, suggesting that it may be possible to construct a manifold that would represent movement of the masticatory system within task relevant dimensions. To accomplish this goal, future EMG studies should be aimed at defining the full range of jaw elevator motor patterns and their relationship to jaw kinematics during various stages of the chewing sequence. Ultimately, the aim should be to identify the extent to which various optimality criteria affect the system (Ross and Iriarte-Diaz 2014). Once the task relevant dimensions have been identified, it will be feasible to design experiments that specifically test the neural factors that influence specific muscle activation patterns via perturbation.

AIM III

Aim III sought to understand how functional groups of muscles are represented within the primary orofacial motor cortex (MIo) of *Macaca Mulatta*. The findings of Aim III suggest that while there is a larger somatotopic gradient as suggested by previous researchers (Huang et al. 1988), specific functional groups of muscles are clustered together spatially within the cortex. Each functional group of muscles produces an etiologically relevant behavior that may be similar between individuals. However, a larger sample size is needed to determine inter-individual variability in these clusters and the extent to which they can be modified based on individual experience.

MIo is an ideal target to better understand how movement is organized in the cortex and the role the cortex plays in generating behavior. There are monosynaptic and bilateral projections between MIo and the motor neurons of the trigeminal, facial, and hypoglossal nuclei. However, chapters I and II study rhythmic mastication while chapter III focuses on MIo, which influences discrete voluntary behaviors. A future course of study may adapt this stimulation protocol to target the cortical masticatory area (CMA) as oppose to MIo. Low frequency long train stimulation of CMA produces rhythmic behaviors like chewing and lapping. Stimulating different points within MIo modifies the behavior/ kinematics observed while modifying the frequency and amplitude of stimulation modifies the rhythm of the behavior (Avivi-Arber et al. 2005).

CONCLUDING STATEMENTS

The present study begins to determine why some motor patterns are more common than others from both a biomechanical perspective and a neural one. With Aim I, we determined that the triplet motor pattern is more common than others. Aim II determined that the triplet motor pattern does not form an invariant unit of control of the CNS. Instead, the CNS recruits a specific motor pattern prior to cycle start based on internal criteria. This motor pattern is continuously modified based on sensory feedback in task relevant dimensions. Variability in dimensions irrelevant to the task is ignored. Finally, in Aim III, we determined that specific functional groups of muscles are clustered together spatially within MIo. This dissertation only begins to deconstruct the problem of why and how the CNS selects specific motor patterns during feeding: many aspects of this problem remain to be explored.

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