
Opinion | Sensory and Motor Systems

Vocal Motor Performance in Birdsong Requires Brain-Body Interaction

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44

45 ***Significance statement***

46 Motor skill learning typically occurs in a period when the brain needs to navigate a body that is still
47 growing and developing. How the changing body, neural circuit formation and motor coding influence
48 each other remains unknown. Songbirds provide excellent model systems to study motor skill
49 learning. It has recently been shown that songbird vocal muscles double in speed during sensorimotor
50 learning. Here we argue that these contractile as well as morphological changes stem predominantly
51 from use and only secondarily from hormones or genetic programs. This implies that muscle training
52 constrains skill learning trajectories. As contractile muscle property changes must require altered
53 motor codes for achieving the same acoustic targets, the final performance results from interactions
54 between brain and body.

55

56 **Introduction**

57 Understanding how novel behaviors are learned remains a major challenge to modern neuroscience.
58 Acquiring and mastering fine motor skills, from dexterity in piano playing to microsurgery or speech,
59 can take weeks to months or even years and is strongly affected by injury, stroke and developmental
60 as well as neurodegenerative disorders. Most fine motor skill learning occurs postnatally from infancy
61 to adolescence when the brain needs to navigate a body that is exhibiting large changes due to
62 growth and development. Changes in neural coding and circuit development remain challenging to
63 follow over meaningful timescales in single individuals and are thus typically studied during rather brief
64 periods in adult subjects (Li et al., 2001; Xiao et al., 2006) or during recovery after injury (Nudo et al.,
65 1996; Dancause et al., 2005). Songbirds however, provide a powerful and unique system to study
66 motor skill learning over ethologically meaningful time scales (Brainard and Doupe, 2013).

67 The brain does not function in isolation. All animal behaviors result from complex system-wide
68 interactions between nervous system, body, and surrounding environment (Chiel and Beer, 1997; Lum
69 et al., 2005; Nishikawa et al., 2007; Tytell et al., 2011; Düring and Elemans, 2016; Sober et al., 2018).
70 Motor pathways produce precisely timed complex sequences of motor commands to activate muscles.
71 The forces ultimately generated by muscles strongly depend on dynamic body motion and
72 environmental conditions through the muscle's nonlinear force-length and force-velocity properties
73 (Düring and Elemans, 2016). Thus, motor control systems are closed loop systems (Roth et al., 2014)
74 and the activity of neural circuits can be understood only by considering the biomechanics of muscles,
75 bodies, and the exterior world (Tytell et al., 2011).

76 How the developing body influences circuit formation and neural coding in the brain and vice versa is
77 still largely unknown (Avitan and Goodhill, 2018). Recent work showed that developmental changes in
78 vocal behavior of marmoset monkeys that were typically attributed to neural changes can be

79 explained by changes in the body (i.e. growth of the lungs, Zhang and Ghazanfar, 2018), emphasizing
80 the need for an embodied view on motor control during vocal development.

81 In this opinion piece, we argue that contractile changes occurring in the vocal muscles of songbirds
82 during song learning stem predominantly from interactions between brain and body. This implies that
83 extensive training of syringeal muscles is essential to achieve their maximal performance and that the
84 duration and trajectory of song learning are not solely set by neural circuit formation. Given that
85 virtually all motor skills or at least their building blocks are acquired during times while the body is still
86 changing, truly understanding motor coding and its pathologies requires rethinking and an embodied
87 approach to understand motor learning.

88

89 ***Songbird brain and body change over vocal development***

90 The sensorimotor phase of song learning in zebra finches takes about two months and starts when
91 juveniles start producing subsong at 28 days post hatching (DPH). Song development proceeds from
92 subsong through plastic song to adult, so-called crystallized song, which is reached around 100 DPH
93 (Roper and Zann, 2006). Over the course of vocal development, a network of discrete interconnected
94 forebrain nuclei dedicated to learn and produce song (hereafter referred to as song system) is
95 changing profoundly in morphology and function. Significant research effort has been dedicated to
96 describing and understanding learning related changes in the brain during vocal development in
97 songbirds (Fee and Goldberg, 2011; Brainard and Doupe, 2013). However, despite our rapidly
98 advancing knowledge of the song system, unfortunately very little is known about the motor code that
99 drives the three main motor systems involved in sound production: the vocal organ – the syrinx –, the
100 respiratory system, and upper vocal tract (Elemans, 2014; Schmidt and Goller, 2016). The motor
101 neurons that control all these muscles are all located in small oblong nuclei in the brainstem (Schmidt
102 and Martin Wild, 2014) and their location and small size complicates chronic recording in freely

103 behaving animals even in adults (Williams and Nottebohm, 1985). At the terminus of the premotor
104 pathway of the song system, *in vivo* recordings show that over vocal development the pre-motor
105 neurons gradually change their firing pattern from highly variable patterns into sparse high-frequency
106 bursts (Oliveczky et al., 2011). In adult males these premotor neurons are precisely locked to song
107 timing (Sober et al., 2008; Oliveczky et al., 2011) and can causally explain variation in biomechanics
108 and behavior of the respiratory motor system (Srivastava et al., 2017).

109 In parallel, the syrinx also exhibits changes during the sensorimotor learning phase. Syringeal muscle
110 mass and cross-sectional area increase from hatching to adulthood and sex differences can be found
111 after 20 DPH in zebra finches (Godsave et al., 2002). However, such morphological changes do not
112 allow reliable inferences about changes in contractile properties of the muscle, such as contraction
113 speed, maximal tension, force-length and force-velocity profiles. Because contractile muscle
114 properties determine the forces that act on body and environment, they are critical traits for
115 understanding the biomechanics of vocal production and thereby linking motor commands to
116 behavioral output, i.e. song. It has recently been shown that over song learning, the superfast
117 syringeal muscles controlling song double in isometric contraction speed and ultimately reach the
118 maximal attainable speed possible in vertebrate synchronous muscle (Mead et al., 2017). The muscle
119 speed increase was associated with a composition change of expressed heavy myosin chain gene
120 isoforms (*MYH*) towards near-exclusive expression of *MYH13* aka superfast myosin (Mead et al.,
121 2017). Concluding, over vocal development syringeal muscles exhibit changes in morphology as well
122 as contractile properties.

123 In the following sections we will review the three most likely factors that could drive could these
124 changes: hormones, an innate developmental program, or neural drive.

125

126 ***Hormonally mediated changes cannot explain adult syrinx dimorphism***

127 In adult zebra finches, the entire song system, including the syrinx exhibits differences between sexes.
128 Most nuclei of the song system are smaller in females (Nixdorf-Bergweiler, 1996; Shaughnessy et al.,
129 2019), females have a smaller motor nucleus projecting to the syrinx (Godsave et al., 2002; Wade et
130 al., 2002), the syrinx itself is also smaller with a less developed skeleton (During et al., 2013),
131 syringeal muscles have less mass (Bleisch et al., 1984; Wade and Buhlman, 2000; During et al.,
132 2013) and a lower contraction speed (Elemans et al., 2008) – albeit still nearly two orders of
133 magnitude faster than locomotory muscles. Moreover, syrinx muscles in females express less *MYH13*
134 than in males (Mead et al., 2017). The sexual dimorphic nature of these observations suggests that
135 these traits are mediated by gonadal steroid hormones.

136 In general, this hypothesis is supported by previous studies showing that the syrinx is sensitive to
137 manipulations of steroid hormone levels: Treating adult females with testosterone increases muscle
138 mass, fiber diameter, and number of acetylcholine receptors, masculinizing the syrinx (Bleisch et al.,
139 1984; Wade and Buhlman, 2000). Similarly, decreasing testosterone levels in males feminizes the
140 syrinx, which is reflected in a loss of muscle mass (Luine et al., 1980; Bleisch et al., 1984; Wade and
141 Buhlman, 2000). However, masculinization never reaches male levels and axotomizing (severing the
142 innervating nerve) the syrinx of testosterone-treated females abolishes masculinization (Bleisch et al.,
143 1984; Wade and Buhlman, 2000), which indicates that sex differences in syringeal properties are not
144 solely driven by steroid hormones. This is further supported by the finding that androgen receptor
145 expression only gets dimorphic after 30 DPH, 10 days after muscle weight and cross-sectional-area
146 start to be different between the sexes (Godsave et al., 2002). Thus, the changes in muscle
147 morphology seem to be driven by the change in neural drive due to the onset of singing activity in
148 males around 28 DPH (Arnold, 1975) rather than direct action of hormones on the syrinx. This
149 hypothesis is further strengthened by the finding that castration changes the normal song learning

150 trajectory only slightly, mostly by decreasing the amount of song per day and delaying crystallization in
151 some but not all animals (Arnold, 1975).

152 Taken together we conclude that steroid hormones are critical but not sufficient to induce and maintain
153 sexual dimorphism of the syrinx.

154

155 ***Muscle use drives morphological and contractile changes***

156 Skeletal muscle functionality is known to change with use in adults or after peripheral nerve damage
157 and use reduction causes muscle atrophy (Buller et al., 1960; Buller et al., 1987; Lømo, 2003;
158 Schiaffino and Reggiani, 2011). As such, the most drastic experiment to investigate neural drive on
159 muscles is axotomy, i.e. to sever the nerve innervating the muscle. Axotomizing the syrinx in
160 songbirds leads to a decrease in fundamental frequency and amplitude of the produced sound and the
161 conversion of all syllables into harmonic stacks due to the loss of fast frequency modulations (Williams
162 et al., 1992; Daley and Goller, 2004; Roy and Mooney, 2007; Secora et al., 2012; Vallentin and Long,
163 2015). Morphologically, syringeal muscles atrophy after axotomy (Urbano et al., 2013), suggesting
164 that usage is required to maintain muscle mass. However, it remains unknown how axotomy affects
165 contractile related changes, such as contraction speed, force-length and force-velocity profiles, *MYH*
166 composition and *MYH13* expression in particular.

167 The discovery of *MYH13* expression in syringeal muscles (Mead et al., 2017) placed them
168 conclusively in the lineage of craniofacial muscles, corroborating earlier developmental studies
169 (Noden et al., 1999; Noden and Francis-West, 2006). These skeletal muscles can uniquely express
170 several rare myosin heavy chain isoforms, among them *MYH13*. The craniofacial muscles also include
171 extraocular and most laryngeal muscles (Briggs and Schachat, 2000; Hoh, 2010) and are
172 characterized by extremely fast force development, reaching maximal tension within a few

173 milliseconds (Elemans et al., 2008; McLoon and Andrade, 2012). *MYH13* has been proposed to be
174 responsible for very high contraction speeds and this notion is strengthened by the unusually high
175 detachment rate from actin (Bloemink et al., 2013).

176 In our opinion, the most likely hypothesis to explain the upregulation of *MY13* and doubling in
177 contraction speed in the syrinx during song learning is that the increased use – or training – of
178 muscles is causally driving these changes. This hypothesis is supported by three lines of evidence:

179 1. *Timescale of upregulation.* In all craniofacial muscles studied to date that express myosin *MYH13*,
180 contraction speed as well as *MYH13* expression increase over a similar, relatively slow (i.e. weeks)
181 time course after birth. In songbirds, *MYH13* expression changes over two months during
182 sensorimotor learning (Mead et al., 2017). In kittens, twitch speed as well as tetanic tension of
183 extraocular muscles increases during the first 20 weeks of life (Lennerstrand and Hanson, 1978). In
184 mouse and rat extraocular muscles and the rat larynx *MYH13* expression increases over the first 20-
185 30 days of life (Shiotani et al., 1999; Zhou et al., 2010; Moncman et al., 2011), but to our knowledge
186 no data on the development of contraction speed is available in these muscles.

187 2. *Neural drive influences muscle speed and MYH expression.* In general, skeletal muscle functionality
188 changes with neural drive. Use reduction causes muscle atrophy (Buller et al., 1960; Buller et al.,
189 1987; Lømo, 2003; Schiaffino and Reggiani, 2011) and transnervation with a faster spiking nerve
190 increases contraction speed (Paniello et al., 2001). Neural stimulation is known to drive *MYH*
191 expression patterns and contraction speed: In rats, neural activity associated with optokinetic and
192 vestibulo-ocular reflexes stimulates *MYH13* expression in extraocular muscles (Brueckner et al., 1999;
193 Moncman et al., 2011). In kittens, the earlier described postnatal increase in contraction speed is
194 impeded by impairing binocular vision (Lennerstrand, 1979; Lennerstrand and Hanson, 1979).
195 Likewise, upregulation of *MYH13* in rodent extraocular and laryngeal muscles is prevented by visual
196 deprivation or axotomizing the larynx, respectively (Brueckner and Porter, 1998; Shiotani and Flint,

197 1998; Brueckner et al., 1999), which in addition provides evidence against the hypothesis that a fixed
198 postnatal developmental program controls *MYH13* upregulation. Last, in all *MYH13* expressing
199 craniofacial muscles, the increase in contraction speed and *MYH13* expression coincides with the
200 onset of muscle use (Lennerstrand and Hanson, 1978; Shiotani et al., 1999; Zhou et al., 2010;
201 Moncman et al., 2011; Mead et al., 2017).

202 **3. Location of *MYH13* transcription.** In particular the expression of *MYH13* opens the possibility that
203 changes in the properties of syringeal muscles are driven by neural activity, as *MYH13* is known to be
204 transcribed close to the neuromuscular junction in extraocular muscles (Briggs and Schachat, 2002).
205 It has been speculated that the electrical or chemical activation of motor neurons directly stimulates
206 *MYH13* transcription linked to acetylcholine receptors (Sanes and Lichtman, 2001; Rubinstein et al.,
207 2004). However, it is unknown to what extent the amount of stimulation affects *MYH13* transcription
208 and other muscle properties.

209

210 ***Conclusions and implications***

211 Taken together the data above strongly suggest that the postnatal increase in muscle speed and
212 *MYH13* expression in the songbird syrinx is primarily caused by the use of the muscles and only
213 secondarily due to hormones and innate genetic programs.

214 We propose this hypothesis has several implications for motor learning:

215 **1. Time course of song learning is set by muscle training.** Because previous work established that
216 *MYH13* expression is regulated by use and correlates to speed, we suggest that extensive use of
217 syringeal muscles is essential to achieve their maximal speed. The timescale of *MYH13* upregulation
218 in craniofacial muscles is more than four weeks in all studied model systems, and about seven weeks
219 in songbirds. Because this duration overlaps the typical temporal trajectory for skeletal muscle

220 endurance or speed training, we speculate that the total duration and trajectory of song learning is not
221 solely set by neural circuit formation but must also be required for muscle training. As female
222 songbirds may be able to perceive millisecond scale variations (Prior et al., 2018) and prefer sped-up
223 song in several species (Draganoiu et al., 2002; Weiss et al., 2012), we further speculate that
224 millisecond fine-scale acoustic modulations, such as frequency modulation or on- and offset precision,
225 can act as an honest signal (Searcy and Nowicki, 2005) for metabolic energy invested in muscle
226 training.

227 **2. Execution error is constrained by immature motor code and muscle speed.** In adults,
228 premotor codes and song behavior occur at millisecond scale precision (Chi and Margoliash, 2001;
229 Hahnloser et al., 2002) and recently variation in spike timing at millisecond timescales has been
230 shown to causally affect biomechanics and behavior (Tang et al., 2014; Srivastava et al., 2017; Sober
231 et al., 2018). In contrast, the plastic phase of song learning is marked by a higher rendition to rendition
232 variability in acoustic parameters like syllable duration and frequency (Ali et al., 2013). This variability
233 is essential for vocal exploration during trial and error learning (Charlesworth et al., 2011) and thought
234 to be driven mostly by the basal ganglia (Kojima et al., 2018). We propose an additional interpretation:
235 Faster muscles allow a more temporally precise actuation to achieve acoustic targets leading to less
236 variability in e.g. duration of syllables. In other words, we assume that muscle speed increase leads to
237 execution error decrease. We hypothesize that the (initially) slower superfast syringeal muscles
238 constrain the precision to execute motor sequences during vocal learning, especially at the start of
239 song ontogeny. We predict that variable vocalizations observed during song learning reflect immature
240 brain circuits as well as muscle speed.

241 **3. Changes in contraction speed require motor code adaptation for achieving the same target.**
242 During motor learning, animals try to minimize error for achieving task-specific motor targets. In the
243 zebra finch the acoustic song template is considered to not change during sensorimotor learning

244 (Mooney, 2009). As contraction speed of syringeal muscles changes over the course of weeks,
245 playing a hypothetical stereotyped motor code will lead to changes in force profiles. Consequently, the
246 acoustic targets, such as amplitude, entropy or fundamental frequency, will also change and in turn
247 lead to changes in error magnitude. Thus, we propose that the observed contractile changes of
248 syringeal muscles require altering the motor code during development to achieve the same force
249 profiles and acoustic targets.

250

251 The hypotheses presented above remain to be tested experimentally. Only with a systems view will
252 we be able to explain and understand the development of complex behaviors. Given the dedicated
253 neural, muscular and genetic substrates, the knowledge accrued to date and the increasing number of
254 genetic tools (from RNA-interference to DREADDs and optogenetics) available to the field, birdsong is
255 an ideal system to embrace such an integrative approach.

256

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