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Tuning of feedforward control enables stable muscle force-length dynamics after loss of autogenic proprioceptive feedback

Authors: JC Gordon¹, NC Holt², AA Biewener³ and MA Daley^{1,4*}

Affiliations:

¹ Comparative Biomedical Sciences, Royal Veterinary College, University of London

² Evolution, Ecology & Organismal Biology, University of California, Riverside

³ Organismic and Evolutionary Biology, Harvard University

⁴ Ecology and Evolutionary Biology, University of California, Irvine

Running head: *muscle dynamics in running after self-reinnervation*

Address for correspondence:

*Author for correspondence: madaley@uci.edu

University of California, Irvine, 1408 Biological Sciences, Irvine, CA 92697-2525

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20 **Abstract**

21 Animals must integrate feedforward, feedback and intrinsic mechanical control mechanisms to maintain stable
22 locomotion. Recent studies of guinea fowl (*Numida meleagris*) revealed that the distal leg muscles rapidly
23 modulate force and work output to minimize perturbations in uneven terrain. Here we probe the role of reflexes
24 in the rapid perturbation responses of muscle by studying the effects of proprioceptive loss. We induced bilateral
25 loss of autogenic proprioception in the lateral gastrocnemius muscle (LG) using self-reinnervation. We
26 compared *in vivo* muscle dynamics and ankle kinematics in birds with reinnervated and intact LG. Reinnervated
27 and intact LG exhibit similar steady state mechanical function and similar work modulation in response to
28 obstacle encounters. Reinnervated LG exhibits 23ms earlier steady-state activation, consistent with feedforward
29 tuning of activation phase to compensate for lost proprioception. Modulation of activity duration is impaired in
30 rLG, confirming the role of reflex feedback in regulating force duration in intact muscle.

31 Main text

32 Introduction

33 Sensory feedback is widely accepted as an integral component of vertebrate locomotor control (Cohen, 1992;
34 Donelan & Pearson, 2004; Grillner, 2011; Prochazka & Ellaway, 2012; Rossignol et al., 2006). Proprioception
35 from muscle mechanoreceptors contributes to 1) short-latency reflexes via spinal mono- and polysynaptic
36 pathways to regulate the ongoing activity and muscle mechanical output (force, stiffness, impedance and work)
37 and 2) longer-latency responses to coordinate and maintain task-level goals for balance and movement (Grillner,
38 2011; Lam & Pearson, 2002; Frigon & Rossignol, 2006; Prochazka & Ellaway, 2012; Proske & Gandevia, 2012;
39 Rossignol et al., 2006; Safavynia and Ting 2013; Sherrington & Laslett, 1903; Sherrington 1910). Proprioceptive
40 reflexes can occur through *autogenic* (self-generated) pathways arising from the same muscle, and through
41 *heterogenic* pathways arising from other muscles via spinal interneurons (Abelew et al., 2000; Frigon &
42 Rossignol, 2006; Lam & Pearson, 2002; Nichols 1989; Ross & Nichols 2009). Thus, the relationship between a
43 specific sensory signal and its resulting effects is complex and dynamic.

44 Despite recognized functions of proprioception, the relative contribution of feedback control in high-speed
45 locomotion remains unclear. Sensorimotor delay constrains how quickly an animal can sense and respond to a
46 stimulus using feedback control (More & Donelan, 2018; More et al., 2010). The fastest possible feedback loop
47 occurs through mono-synaptic reflexes, which involve a delay that increases in proportion to nerve transmission
48 distance. This reflex delay becomes a larger fraction of the stride cycle with increasing speed, limiting time
49 available for reflex-mediated correction.

50 The challenges of long delays relative to stride cycle times likely necessitates greater reliance on feedforward
51 control strategies at higher speeds. Here we use feedforward to refer to the contributions to motor output arising
52 from the motor cortex, descending pathways and rhythmic spinal networks (Frigon & Rossignol, 2006; Pearson,
53 2000; Yakovenko et al. 2004). Rhythmic spinal networks can generate the basic flexion and extension motor
54 pattern for gait, even when proprioceptive feedback is removed (e.g., Pearson et al., 2003; Sharp & Bekoff
55 2015). Normally, however, descending networks act in concert with spinal networks and feedback, using
56 multimodal and distributed sensory inputs to update state estimates, regulate rhythm and control foot placement
57 (Cohen 1992; Drew and Marigold 2015; Marigold and Drew 2017; Pearson et al. 1999; Potocanac et al. 2014;
58 Todorov 2004; Wagner & Smith 2008; Wolpert et al. 2011). Consequently, there is no ‘pure’ feedforward control
59 within vertebrate systems. We use the term here as a pragmatic distinction, where *feedforward* refers to
60 anticipatory ‘look-ahead’ control over one or more stride cycles, and *feedback* refers to reflex-mediated reactive
61 responses to perturbations.

62 Although feedforward networks normally act in concert with feedback, feedforward motor activation coupled to
63 intrinsic muscle properties can be sufficient to produce stable gait (Yakovenko et al., 2004). Consistent with this,
64 the lateral gastrocnemius (LG) of guinea fowl (*Numida meleagris*) rapidly absorbs energy in response to

unexpected drop perturbations (Daley et al., 2009), stabilizing high speed running without a reflex response. The rapid perturbation response arises from the intrinsic mechanical properties of the muscle-tendon tissues and musculoskeletal system (Brown & Loeb 1999; Loeb et al. 1999; Jindrich and Full 2002). Intrinsic mechanical responses can be actively tuned by the specific feedforward pattern of muscle activation. For example, humans hopping on surfaces with randomized, sudden increases in ground stiffness show a feedforward increase in muscle co-activation and knee flexion, increasing mechanical stability (Moritz & Farley 2004). However, many perturbation responses involve multiple control mechanisms that overlap in time. Guinea fowl running over obstacles use a combination of feedforward, intrinsic mechanical and reflex-mediated mechanisms, with a delay of ~40 ms for reflex-mediated increases in muscle force (6ms reflex latency + 34ms force development delay: Daley et al. 2009; Daley & Biewener, 2011). Considering that the feedforward and intrinsic mechanical contributions alter ongoing muscle dynamics *before* the reflex-mediated response, it is difficult to disentangle the specific contributions of proprioceptive reflexes to the observed perturbation responses (Daley & Biewener, 2011; Gordon et al. 2015).

Investigating the role of proprioception through self-reinnervation

Here we probe the integration of feedforward, feedback and intrinsic mechanical control by eliciting a proprioceptive deficit in the lateral gastrocnemius muscle (LG) of the guinea fowl (*Numida meleagris*) using bilateral self-reinnervation (Figure 1). Self-reinnervation involves peripheral nerve branch transection and immediate repair, resulting in recovery of motor output with long-term, local loss of autogenic muscle proprioception (Cope et al., 1994; Bullinger et al. 2011). Self-reinnervation occurs through axonal regrowth and reconnection with denervated tissues over a recovery period of 4-8 weeks (Carr, et al., 2010; Cope, et al., 1994; Gordon & Stein, 1982; Vannucci et al., 2019). Reinnervated muscles retain a deficit in the monosynaptic stretch reflex due to synaptic retraction of primary muscle spindle afferents and disconnection from parent motoneuron populations (Alvarez et al., 2011; Brandt et al. 2015). However, intermuscular force and length feedback networks may remain partially intact (Lyle et al. 2016). Cats and rats with reinnervated muscles maintain whole-limb function by adjusting inter-joint coordination and muscle co-activation to compensate for loss of reflex-mediated ankle stiffness (Abelew et al., 2000; Maas et al., 2007; Chang et al. 2009; Boeltz et al. 2013). These findings highlight the ability of animals to flexibly exploit musculoskeletal plasticity to maintain function and suggest self-reinnervation as a promising tool to investigate sensorimotor control mechanisms.

Studying neuromuscular control in the guinea fowl, a bipedal animal model, provides insight into similarities and differences among vertebrates that may relate to locomotor modality, evolutionary history, or both. Birds share features of sensorimotor structure and function with mammals, including muscle tissue properties (Nelson, et al., 2004; Poore et al., 1997) and muscle proprioception through muscle spindle and Golgi tendon organs (Dorward, 1970; Haiden & Awad 1981; Maier, 1992). Ground birds use bipedal walking and running gaits with mechanics and energetics similar to human locomotion (Heglund, et al., 1982; Taylor, et al., 1982; Gatesy & Biewener, 1991; Roberts, et al., 1997; Daley & Birn-Jeffery, 2018). Bipedal gaits involve substantial periods of

100 single-limb contact, which limits the redundancy of balance mechanisms and poses a challenge for stability
101 (Daley, et al., 2009; Clark & Higham, 2011; Daley & Biewener, 2011). Whereas a quadrupedal cat or rat might
102 be able to compensate for deficits by shifting weight bearing among legs, a biped with a bilateral proprioceptive
103 deficit cannot. Accordingly, one goal of the current study is to explore whether or not guinea fowl exhibit a
104 similar response to proprioceptive deficit as observed in quadrupedal vertebrates (Abelew et al., 2000; Maas et
105 al., 2007; Chang et al. 2009; Boeltz et al. 2013)

106 We hypothesize that autogenic proprioceptive deficit will lead to increased reliance on feedforward control
107 mechanisms and intrinsic muscle mechanics to maintain stable locomotion. To test for shifts in stability and
108 control mechanisms, we measured ankle kinematics and *in vivo* LG muscle dynamics (length, force and
109 activation) during treadmill running on level and obstacle terrain. There are several potential mechanisms to
110 compensate for autogenic proprioceptive deficit: 1) Birds might compensate for proprioceptive deficit by
111 increasing feedforward muscle activation before obstacle contact, as observed in birds negotiating high-contrast
112 obstacles (Gordon et al. 2015). 2) Alternatively, if feedback regulation of LG is essential for stability in fast
113 locomotion, loss of autogenic proprioception may necessitate increased reliance on heterogenic reflex pathways
114 from synergists, with a slight delay compared to intact animals, as suggested by work in cats and rats (Boeltz et
115 al. 2013; Lyle et al. 2016). 3) Finally, if intrinsic mechanics are mainly responsible for the modulation of muscle
116 force and work, we might expect minimal change in muscle activity patterns (EMG), as observed in birds
117 subjected to unexpected drop perturbations (Daley, et al., 2009). We expect birds to compensate for
118 proprioceptive deficit by tuning gait and feedforward muscle activity to maintain a stable response to obstacle
119 perturbations, as observed in reinnervated rats and cats walking on slopes (Abelew et al., 2000; Maas et al.,
120 2007; Chang et al. 2009; Boeltz et al. 2013). If stability is impaired following reinnervation, this should be
121 evident from increased variance and longer time to recover from obstacles. By investigating the shifts in guinea
122 fowl LG muscle force, length and activation dynamics following reinnervation, we hope to gain insight into the
123 mechanisms of sensorimotor integration and plasticity that enable robustly stable and agile bipedal locomotion.

124

125 **Results**

126 **Mechanical function of intact *versus* reinnervated LG**

127 We find that many features of the steady-state *in vivo* mechanical function of the guinea fowl reinnervated lateral
128 gastrocnemius (rLG) are similar to that previously measured in the intact LG (iLG). In Figure 2, average
129 trajectories (mean±95% confidence interval) are shown for muscle strain, force and electromyographic activity
130 (iLG at top, rLG below), with the average for steady level running in grey. During the swing phase of the stride
131 cycle, both iLG and rLG exhibit a period of passive stretch, followed by rapid shortening. Activation and force
132 development begin in late swing around the time of the transition from stretch to shortening, initiating rapid
133 active shortening until foot contact (Figure 2, triangles). At the time of foot contact, force increases rapidly to a
134 peak before midstance, then declines more slowly. Typically, in level running both iLG and rLG show a near-
135 isometric phase in early stance, followed by shortening in late stance, which produces net positive work, as
136 indicated by counter-clockwise force-length work loops (Figure 3). The average magnitude of work output
137 (W_{net}) during steady level running is similar between the iLG and rLG, with similar spread of the distribution
138 around the mean (Figure 4). However, rLG shows faster shortening velocity at peak force (V_{pkF}) compared to the
139 iLG across both level and obstacle terrains (Figure 4), indicating a difference in steady state contraction
140 dynamics.

141 **Force-length dynamics and work output during obstacle negotiation**

142 In obstacle encounters (Figure 2, S 0), foot contact with the obstacle occurs earlier in the stride cycle compared
143 to level terrain, altering force-length dynamics during the obstacle stance period. During obstacle contact, both
144 iLG and rLG remain at longer lengths, force increases rapidly to reach a higher peak force, and the muscle
145 shortens throughout force development, producing positive work (Figure 2, S 0). Both iLG and rLG exhibit
146 increased force and work output in obstacle strides compared to level strides (Figure 3, S 0). The magnitude of
147 the shift in work output (W_{net}) in obstacle strides (S 0) is similar between intact and reinnervated cohorts,
148 increasing by $3.60 \pm 0.57 \text{ Jkg}^{-1}$ in iLG and $3.88 \pm 0.60 \text{ Jkg}^{-1}$ in rLG (mean±95% ci, Figure 4B, Table 2).

149 Although the magnitude of the shifts in work output are similar between iLG and rLG, the mechanisms
150 underlying the shift in work output differ between them (Figure 4). In iLG, increased work upon obstacle contact
151 occurs through modest increases in both peak force (F_{pk}) and shortening velocity (V_{pkF}), compared to level
152 strides. In contrast, rLG exhibits a substantially larger increase in F_{pk} on obstacle strides and maintains similar
153 V_{pkF} between level and obstacle terrain strides (S 0, Figure 4B, Table 2). Reinnervated LG also exhibits small
154 but significant increases in F_{pk} in the strides preceding and following obstacle contact (S-1 and S+1,
155 respectively), compared to level terrain.

156 **Shifts in activation patterns between intact and reinnervated LG**

157 Despite deficits in LG monosynaptic reflex following reinnervation, rLG and iLG show similar increases in total
158 muscle activation intensity (E_{tot} , integral of EMG) in obstacle strides compared to steady level strides (S 0,
159 Figure 5). Intact LG exhibits a 4% increase in duration in obstacle strides (S 0) compared to level; however,
160 there is no significant increase in EMG duration for rLG in S 0 (Figure 5B, Table 2). This suggest that the
161 observed increase in E_{tot} in rLG obstacle strides occurs through increased activation amplitude, not increased
162 duration.

163 Several results suggest a shift in central drive and feedforward activation pattern in rLG compared to iLG.
164 Reinnervated LG exhibits longer steady-state duration of activity (E_{dur}) compared to iLG across all level and
165 obstacle terrain strides, averaging 37% of the stride cycle in rLG compared to 29% in iLG (Figure 5B, Table 2).
166 Additionally, rLG exhibits higher average frequency of EMG activity across all strides compared to the intact
167 cohort (Figure 5, Table 2, Figure 5-figure supplement 1). Finally, the steady-state timing of rLG activation is
168 phase-shifted to 6% (23ms) earlier in the stride cycle relative to the length trajectory, quantified by the variable
169 ‘ E_{phase} ’ (Figure 6, Table 1-2). Earlier activation onset may help explain the higher rate of shortening in rLG
170 compared to iLG, reported above.

171 **Timing of obstacle-induced changes in EMG activity in iLG and rLG**

172 To explore the timing of obstacle-induced shifts in EMG activity relative to perturbations in force and length, we
173 calculated a difference trajectory between the steady-state level and obstacle perturbed stride cycles (S 0 – Lev)
174 for each individual, and then calculated the mean and 95% confidence interval across individuals (Figure 7).
175 Increased EMG activity begins ~30-40 ms *before* obstacle-induced increases in length and force, for both iLG
176 and rLG, suggesting an anticipatory (feedforward) contribution to increases in E_{tot} (Figure 7, arrows indicating
177 ‘anticipatory increase’). In iLG, the anticipatory increase in EMG starts ~25% of stride period (asterisk in EMG
178 trace and vertical dashed line in Figure 7A). Starting around 58% of stride period, there is another distinct burst
179 of increased activity, suggesting reflex-mediated contribution to increased EMG in obstacle strides (Figure 7,
180 arrow indicating ‘reflex’). In rLG, the ‘anticipatory increase’ in EMG starts around 21% of stride (asterisk and
181 vertical dashed line in 7B); however activity in the latter half of stance is highly variable and idiosyncratic
182 among individuals (Figure 7-figure supplement 1), as indicated by the wide 95% confidence intervals spanning
183 the region of time where the iLG shows a distinct reflex response (compare Figure 7B versus 7A lower panels).
184 Cross-correlation between the obstacle perturbation trajectories for iLG reveals a correlation of 0.82 between
185 length and EMG deviations, and a correlation of 0.85 between force and EMG deviations. For rLG, the cross-
186 correlations are reduced to 0.53 between length and EMG deviations, and 0.57 between force and EMG
187 deviations, respectively. The reduced correlations suggest a disrupted reflex-mediated response to muscle load
188 and strain in the latter half of stance in rLG, but which is present in iLG (Figure 7). Considering that the changes
189 in muscle length and force are strongly correlated with each other during obstacle encounters in both intact and
190 reinnervated conditions (Figure 7), it is difficult to distinguish the specific sensory signal eliciting reflex
191 responses.

192 **Stability and kinematic changes during obstacle negotiation in intact vs reinnervated birds**

193 Compared to the intact cohort, birds with rLG exhibit more pronounced shifts in gait dynamics in obstacle
194 terrain relative to level terrain. Obstacle-induced increases in peak force (F_{pk}) are larger for rLG compared to
195 iLG (S 0 Figure 4, Table 2), reflecting larger deviations from steady state in response to the same obstacle.
196 Additionally, rLG shows small but significant increases in peak force (F_{pk}) in the strides preceding and following
197 obstacle contact (S -1, S +1) compared to steady level strides (Figure 4B, Table 2). Multiple significant
198 differences from level strides occur for rLG in S +1, including a $39 \pm 22\%$ increase in E_{tot} , a $6 \pm 2\%$ decrease in
199 force duration and a $4 \pm 3\%$ decrease in stride duration (mean $\pm 95\%$ ci, Table 2). In contrast, most variables for
200 iLG have recovered to steady state in S +1 (Table 2). Both iLG and rLG rapidly increase work output during
201 obstacle encounters and face increased activation costs for locomotion in obstacle terrain. However, rLG shows
202 larger deviations from steady state, and a slower recovery to steady state mechanics and activation level
203 compared to iLG, indicating reduced stability.

204 Reinnervated birds also show differences in running kinematics in obstacle terrain compared to the intact cohort,
205 undergoing a more pronounced increase in ankle flexion in obstacle encounters (Figure 8). Reinnervated birds
206 also use a shorter stride duration immediately preceding the obstacle encounter (S -1), suggesting anticipatory
207 preparation that is not observed in intact birds (Figure 8). These observations suggest reduced ankle stiffness and
208 increased anticipatory preparation for obstacle encounters in the reinnervated birds.

209

210 Discussion

211 What is the role of proprioception in the control of high-speed locomotion?

212 We investigated the role of reflexes in the sensorimotor control of running by examining the effects of proprioceptive
213 deficit on the mechanical function of the lateral gastrocnemius muscle (LG) of guinea fowl. Long sensorimotor delays
214 relative to limb cycling times necessitate that animals use a combination of feedforward, feedback and intrinsic
215 mechanical control mechanisms to achieve stable locomotion at high speeds (Brown & Loeb, 2000; Jindrich and Full
216 2002; Daley & Biewener, 2011; Daley et al., 2009; Frigon & Rossignol, 2006; Grillner, 2011; Lam & Pearson, 2002;
217 More & Donelan, 2018; Pearson & Gramlich, 2010; Prochazka & Ellaway, 2012). We hypothesized that an autogenic
218 proprioceptive deficit will lead to increased reliance on feedforward tuning of muscle activity to achieve stable muscle
219 dynamics in obstacle terrain. In birds with intact LG proprioception, the timing of muscle activity in obstacle-perturbed
220 strides is consistent with combined feedforward and feedback control (Daley & Biewener 2011, Gordon et al. 2015).
221 Birds with reinnervated LG (rLG) exhibit a consistent phase shift in EMG onset relative to muscle length, with activation
222 starting 6% earlier (23ms) in the steady state contraction cycle, in both level and obstacle terrain (Figure 6, Table 2).
223 This is consistent with a feedforward tuning of rLG activation timing to enable rapid force development and high muscle
224 stiffness during stance, in the absence of monosynaptic reflexes. Regulation of EMG duration in obstacle strides (S 0) is
225 absent in rLG (Figure 5), suggesting that proprioceptive feedback in late stance normally regulates force duration, which
226 is disrupted following reinnervation.

227 A stable intrinsic mechanical response with neither feedforward- nor feedback-mediated changes in neural drive
228 can occur when a perturbation is encountered at high running speeds (Daley et al. 2009). Rapid changes in
229 muscle length and velocity in response to perturbations can decouple activation and force development (Daley et
230 al. 2009, Daley & Biewener, 2011). In our previous work on intact *in vivo* muscle dynamics, variation in LG
231 muscle strain during initial foot contact and limb loading explained 60% of the variation in force developed in
232 obstacle encounters, while variation in LG muscle activation explained only 9%. This clearly demonstrates the
233 decoupling between activation and force development that can occur *in vivo* (Daley & Biewener, 2011). These
234 intrinsic mechanical effects minimize the disturbances in body dynamics that arise from terrain height
235 perturbations, enabling rapid recovery to steady gait. Similar intrinsic mechanical stabilizing responses have
236 been demonstrated in the distal hindlimb joints of hopping and running humans subjected to unexpected changes
237 in terrain height and stiffness (Dick, et al., 2019; Ferris, et al., 1999; Moritz & Farley, 2004). In concert with the
238 stabilizing contributions of the intrinsic muscle-tendon dynamics, guinea fowl with intact proprioception also use
239 feedforward and feedback regulation of muscle activity to maintain stability in obstacle terrain, with greater
240 feedforward contributions when obstacles are visible and high contrast (Daley & Biewener, 2011, Gordon et al.
241 2015).

242 We find that guinea fowl with LG proprioceptive deficit achieve similar increases in total EMG activity during
243 obstacle strides; however, the increases in activity occur early in the stride, before obstacle-induced changes in

244 muscle force and length (Figure 7). This is consistent with anticipatory, feedforward increases in neural drive to
245 the muscle, as observed in birds running over high-contrast visible obstacles (Gordon et al. 2015), and humans
246 hopping on randomized but expected increases in surface stiffness (Moritz & Farley, 2004). These findings are
247 consistent with a hybrid feedforward/feedback control model as conceptualized by Kuo (2002) in which
248 feedforward and feedback gains are balanced to enable accurate state estimation and robust cyclical dynamics in
249 the presence of both disturbances and sensory error. Although the reinnervated LG contributes to an effective
250 obstacle negotiation response, it requires a longer recovery time and increased muscle activity following obstacle
251 contact (Figure 5B). This suggests that the integrated response of the intact neuromuscular system enables robust
252 stability with lower muscle activation costs.

253 Several features of the kinematics and muscle dynamics suggests coordinated plasticity and tuning of feedforward
254 control to compensate for reflex deficit following recovery from nerve injury (Figure 9). We observe similar
255 increases in work output in response to obstacle encounters in rLG and iLG (Figure 4B). This finding is
256 consistent with the idea that muscle work modulation is an important feature of task-level control for stability in
257 uneven terrain (Daley et al. 2009; Daley & Biewener 2011). However, work modulation is achieved through
258 different underlying mechanisms in rLG and iLG. Earlier steady state activation in rLG (lacking autogenic
259 proprioceptive feedback) enables higher muscle force development in early stance to resist the external load
260 applied at foot contact, which likely contributes to the higher rate of shortening throughout stance. Additionally,
261 rLG exhibits larger increases in peak force in obstacle encounters compared to iLG (Figure 4B). This increase in
262 peak force likely involves both active and passive components: an active contribution from increased
263 feedforward drive and EMG amplitude (Figure 5B), and a passive contribution from increased stretch of
264 connective tissues associated with a more flexed ankle posture at foot contact (Figure 8). Finally, Birds with rLG
265 also show an anticipatory shift in stride duration before obstacle encounters, which is not observed in the intact
266 cohort (Figure 8B) and may help control landing conditions for obstacle encounters (Gordon et al. 2015). These
267 findings suggest that reinnervated birds achieve effective muscle work modulation and stable obstacle
268 negotiation through feedforward tuning of muscle activation and gait to compensate for loss of autogenic
269 proprioception.

270 A recent study by Sawicki et al. (2015) found that earlier onset of activation was associated with a shift to energy
271 absorption in cyclical muscle contractions with a sinusoidal MTU length trajectory. We find here that earlier onset is
272 associated with greater shortening and work production. The specific response of a muscle to a shift in activation phase
273 is likely to be highly sensitive to the specific steady-state length trajectory of the muscle. Muscle force capacity and the
274 activation and deactivation kinetics are substantially influenced by velocity and recent strain history, as demonstrated in
275 controlled studies of *in vitro* muscle force-length work loops (Askew & Marsh, 1998; Josephson, 1999). Further work is
276 needed to understand how *in vivo* muscle fascicle length dynamics interact with neural activation patterns and MTU
277 compliance to enable tuning of muscle contraction dynamics to the mechanical demands of cyclical locomotor tasks.

278

279 We do observe shifts in muscle activity in late stance in some reinnervated individuals in response to obstacle
280 encounters, which suggests heterogenic reflex responses in reinnervated LG (Figure 2). However, these
281 responses are variable and idiosyncratic, with some birds showing increased EMG in late stance in obstacle
282 strides, and others showing a decrease (Figure S3). The variable and idiosyncratic reflex responses result in
283 wide confidence intervals for the obstacle-induced EMG response in the latter half of stance, despite consistent
284 force-length trajectories over the same time-period (Figure 7). Idiosyncratic use of heterogenic reflex modulation
285 across individuals following nerve injury recovery is consistent with findings in cats (Lyle et al. 2016; Lyle &
286 Nichols, 2018). Guinea fowl have several agonist muscles to the LG that could contribute to heterogenic
287 feedback modulation, including the medial gastrocnemius and digital flexors (Daley & Biewener, 2011, Gordon
288 et al. 2015). However, no muscle is an exact synergist of the LG, because each has a unique combination of
289 moment arms, fiber length, pennation angle and connective tissue compliance (Daley & Biewener 2003; Cox et
290 al. 2019). Consequently, it is unlikely that proprioception from agonists can completely restore accurate sensing
291 to regulate LG force and work output. Additionally, in the presence of increased sensory error and noise, birds
292 may learn over time to compensate through sensory integration in higher CNS pathways, leading to updated
293 central coordination and feedforward drive to rhythm generating networks.

294 It was previously unknown whether guinea fowl would respond to reinnervation and proprioceptive deficit in a
295 manner similar to quadrupedal mammals. We find that our results are consistent with the findings on rats and
296 cats. Reinnervated cats and rats exhibit shifts in feedforward muscle activity and inter-joint coordination during
297 slope walking, to compensate for loss of reflex-mediated ankle stiffness (Abelew et al., 2000; Maas et al., 2007;
298 Chang et al. 2009; Boeltz et al. 2013). Cats and rats also preserve task level features of gait, such as leg length
299 and body motions, despite variance in muscle and joint dynamics (Chang et al. 2009; Boeltz et al. 2013). These
300 findings suggest performance of task-level goals as a target of sensorimotor optimization. Also similar to cats,
301 guinea fowl exhibit variation among individuals in heterogenic compensation for loss of the autogenic stretch
302 reflex, as suggested by the variable tendon tap responses and variation in late-stance EMG activity in obstacle
303 perturbed steps (Figure 7; Figure S3). Work on cats suggests complex intermuscular feedback connectivity,
304 which can recovery to varying degrees following reinnervation (Cope et al. 1994; Pearson et al. 1999; Lyle et al.
305 2016, Lyle and Nichols 2018). This complexity and variability among individuals following recovery from nerve
306 injury reflects the complexity and plasticity of proprioceptive feedback networks. Nonetheless, the recovery of
307 consistent task-level mechanical function supports the idea that sensorimotor control is optimized to maintain
308 task-level performance goals such as stable body dynamics (Chang et al. 2009; Safavynia and Ting 2013).

309 **Limitations and future directions:**

310 One of the major limitations in the current study is the potential for multiple differences between the intact and
311 reinnervated cohorts that were not controlled, because the experiments on the two cohorts were conducted over
312 different periods of time. The self-reinnervation procedure requires a long-term recovery period and results in a
313 chronic sensory deficit, which is likely to lead to a complex array of changes in the musculoskeletal tissues and

the sensorimotor networks. The current study did not include a sham-surgery experimental control, and we did not strictly monitor the ages of the original intact cohort at the time of the *in vivo* muscle-tendon surgeries. Nonetheless, it is reassuring that our findings are consistent with similar studies in cats and rats, suggesting feedforward tuning of muscle activation and ankle kinematics to maintain stability following loss of proprioception. In future experiments, it will be important to control the timing and amount of exercise training in intact and reinnervated experimental groups, considering the potential for exercise to influence the recovery process (Boeltz et al. 2013; Brandt et al. 2015).

The recovery process almost certainly involves coupled changes across multiple systems, including connective tissue compliance, muscle activation kinetics, fiber type distribution, motor unit size and distribution, spinal intraneuronal connectivity, and sensory integration in higher CNS centers for state estimation and movement planning. Due to the complex nature of these adaptations, it is challenging to fully tease apart individual contributions and mechanisms from *in vivo* experimental measures alone. In future studies, the coordinated mechanisms of sensorimotor adaptation and plasticity could be systematically explored through a combination of integrative experimental and computational approaches. These approaches could include 1) closed loop neuromechanical simulations to enable predictive hypothesis testing (Ijspeert, 2014; Roth, et al., 2014), 2) combined use of *in vivo* measures of muscle dynamics with *in vitro* testing of muscle contractile dynamics, to replicate biologically realistic force-length contraction dynamics, 3) histological studies to examine changes in muscle fiber type distribution and connective tissue characteristics following reinnervation, and 4) perturbation approaches that probe both short and long term adaptation processes.

It remains unclear how the specific length trajectory and velocity features of *in vivo* muscle dynamics contribute to the intrinsic stability and control of movement. Dynamic measurement techniques are needed to address this challenge and to develop realistic models for *in vivo* muscle-tendon function. In addition to widely recognized force-length and force-velocity ‘Hill-type’ properties, muscle exhibits short and long-term history-dependent changes in force capacity in response to stretch and shortening (Edman, 1975; Edman et al. 1978; Edman, 1980; Josephson, 1999; Herzog, 2004; Edman 2012; Herzog 2014; Rode et al. 2009; Nishikawa et al. 2012; Yeo et al. 2013; Nishikawa, et al., 2018). Recent developments in biorobotic platforms that enable controlled muscle experiments with realistic loading and length trajectories are promising tools for advancing our understanding of the role of intrinsic muscle dynamics in the control of movement (Clemente & Richards, 2012; Richards, 2011; Robertson & Sawicki, 2015). Integrative neuromechanical studies using multiple techniques will be essential for unravelling mechanisms of muscle function, sensorimotor integration and plasticity. Findings from these studies have important implications for many human health conditions, including acute nerve injury, diabetic neuropathy, neurodegenerative disorders, cerebral palsy, and muscular dystrophies.

347 **Materials and Methods**

348 **Animals and treadmill training**

349 We obtained and reared six hatchling guinea fowl keets (*Numida meleagris*) from a breeder (Hidden Hollow
350 Acres, Whitehouse Station, NJ), to allow re-innervation surgeries in juveniles with at least 12 weeks for recovery
351 before *in vivo* muscle procedures (see below). At the time of the *in vivo* muscle measurements, the guinea fowl
352 had reached adult size, averaging 1.81 ± 0.28 kg body mass (mean $\pm$ S.D.). Birds had primary feathers clipped and
353 were trained to run on a level motorized treadmill (Woodway, Waukesha, WI, USA). Training sessions were 15-
354 20 minutes in duration, with breaks for 2 minutes as needed. All experiments were undertaken at the Concord
355 Field Station of Harvard University, in Boston (MA, USA), and all procedures were licensed and approved by
356 the Harvard Institutional Animal Care and Use Committee (AEP #20-09) in accordance with the guidelines of
357 the National Institutes of Health and the regulations of the United States Department of Agriculture.

358 We also include data previously reported in Daley and Biewener (2011) from intact individuals ($n=6$,
359 1.77 ± 0.63 kg body mass) to serve as a control group for statistical comparison to the new dataset ($n=6$
360 reinnervated individuals). We re-analyzed the intact cohort dataset alongside the reinnervated cohort, to ensure
361 consistency in data processing and statistics. The intact data includes a larger sample than reported in Daley and
362 Biewener (2011), because the analysis here includes all strides in the level and obstacle terrain collected for
363 running speeds between 1.3 - 2.0 ms^{-1} . Trials were recorded only for speeds that each bird could comfortably
364 maintain on the treadmill belt for at least 30 seconds, allowing for 10-minute rest periods between trials with
365 access to food and water. We focus on running speeds ($>1.3 \text{ms}^{-1}$), to avoid the confounding effect of different
366 sensorimotor control strategies in walking *versus* running (Gordon et al. 2015). Due to variation among
367 individuals in the successful trials recorded, the intact cohort dataset includes a wider speed range (1.3 - 2.0 ms^{-1})
368 than the reinnervated cohort dataset (1.7 - 2.0 ms^{-1}). However, the analysis is focused on obstacle perturbations
369 compared to steady gait at the same speed, and the datasets include comparable samples of obstacle encounters
370 between the two cohorts: 128 for intact and 133 for reinnervated birds, respectively. In total, the dataset includes
371 1027 strides for reinnervated and 1512 strides for intact individuals and excludes 81 strides as outliers that were
372 non-obstacle encounter strides with Z-score > 4 . No obstacle encounter strides were excluded as outliers. The
373 complete datasets for reinnervated and intact cohorts are available through DataDryad.org, including metadata
374 and Matlab processing scripts (Daley et al. 2020, <https://doi.org/10.7280/D11H49>).

375 **Anesthesia and post-operative care**

376 Birds were induced and maintained on a mid-plane of anesthesia using isoflurane (2 - 3%, mask/intubation
377 delivery). We administered perioperative enrofloxacin and flunixin intramuscularly for analgesia after induction

378 and continued for three days after each surgery. Birds recovered to bilateral weight bearing within 20 minutes
379 following completion of surgical procedures.

380 **Reinnervation surgery**

381 The timing of surgeries was planned based on a pilot study, which found full recovery of LG motor activity by 6
382 weeks following reinnervation surgeries, and continued absence of calcaneal tendon reflex one year later,
383 indicating continued absence of autogenic stretch reflexes (Carr et al., 2010). We bilaterally transected and
384 immediately repaired the peripheral nerve branch supplying the LG muscle in maturing guinea fowl between 7-
385 12 weeks of age. We allowed time for full reinnervation recovery of motor output and growth to adult size
386 before a subsequent surgery to implant muscle transducers (Figure 1). In the reinnervation surgery, a lateral
387 incision was made posterior-distal to the knee to expose the underlying muscle. Blunt dissection enabled
388 exposure and identification of relevant nerve branches, and the identity of the correct nerve branch was
389 confirmed using an isolated nerve stimulator (SD48, Grass Instruments, Warwick, RI) to visualize contraction in
390 the LG. After pre-placement of single longitudinal throw of 6-0 braided non-absorbable silk (Silk, Ethicon,
391 Somerville, NJ, USA) through a 3 mm nerve section, we transected the nerve branch and sutured to appose the
392 cut nerve endings. Fibrin glue (bovine thrombin in CaCl_2 , fibrinogen, fibronectin from bovine plasma) was
393 applied over the apposed nerve endings as an additional repair scaffold (Carr et al., 2010; Spotnitz, 2010). We
394 closed the fascia and skin with 3-0 braided absorbable polyglactin (Vicryl, Ethicon, Somerville, NJ, USA).

395 In the immediate post-operative period, bilateral limb posture was visibly more crouched compared to ‘intact’
396 birds and Achilles tendon tap revealed no stretch reflex response. LG atrophy was qualitatively observed during
397 the first 2 weeks of the recovery period. Within 1 week of surgery, bird activity levels appeared comparable to
398 intact conspecifics, with limb posture partially recovered. From 2-3 weeks onwards, differentiating reinnervated
399 from ‘intact’ birds was not possible from grossly observable limb morphology, posture and gait. We conducted
400 regular treadmill training from 7 weeks after reinnervation surgery. Exercise was started at 7 weeks to ensure
401 synaptic withdrawal of primary afferents before recovery, eliciting a proprioceptive deficit in the self-
402 reinnervated LG. In rats, synaptic withdrawal and resulting proprioceptive deficit is minimized if training is
403 initiated on the 3rd day after nerve injury (Boeltz et al. 2013; Brandt et al. 2015). During training and
404 experiments, birds did not stumble or fall with noticeably greater frequency than observed in intact birds and
405 were able to maintain treadmill position over a similar speed range. At the time of muscle recordings, Achilles
406 tendon tap revealed variable latencies of $49 \pm 43 \text{ms}$ (mean $\pm$ S.D., range 10.3-91.5ms). This may reflect variable
407 recovery of intermuscular reflex connectivity, consistent with observations in cats and rats (Boeltz et al. 2013;
408 Brandt et al. 2015; Lyle et al. 2016). In comparison, the Achilles tendon tap reflex latency in intact birds was
409 $6.1 \pm 1.2 \text{ms}$ (mean $\pm$ S.D), consistent with the mono-synaptic stretch reflex (Nishikawa et al. 2007; Daley and
410 Biewener 2011).

411 **Transducer implantation surgery**

412 When the birds were 23-28 weeks old (13-16 weeks following bilateral reinnervation surgeries), we performed a
413 2nd surgery for transducer placement, following similar procedures as Daley and Biewener (2003). The surgical
414 field was plucked of feathers and gently cleaned with antiseptic solution (Prepodyne, West Argo, Kansas City,
415 MO, USA). We tunneled transducer leads subcutaneously from a 1–2 cm incision over the synsacrum to a
416 second 4–5 cm incision over the lateral left shank. Sonomicrometry crystals (2.0 mm; Sonometrics Inc., London,
417 Canada) were implanted into the lateral head of the gastrocnemius (LG) along the fascicle axis in the middle
418 1/3rd of the muscle belly. Crystals were placed in small openings using fine forceps, approximately 3–4 mm
419 deep and 15 mm apart. We verified signal quality using an oscilloscope and secured the crystals by closing the
420 overlying muscle fascia and lead wires with separate 4-0 silk sutures (Silk, Ethicon, Somerville, NJ, USA). Next
421 to the crystal pair, we implanted bipolar EMG electrodes constructed from two strands of 38-gauge Teflon-
422 coated stainless steel (AS 632, Cooner Wire Co., California, USA) with staggered 1 mm exposed regions spaced
423 1.5 mm apart. Electrodes were placed using sew-through methods and surface silicon anchors (3 x 3 x 2 mm)
424 positioned with a single square knot at the muscle surface-electrode interface (Deban & Carrier, 2002). An “E”-
425 type stainless-steel tendon buckle force transducer insulated with a polyurethane coating (Micro-Measurements,
426 Raleigh NC) was implanted on the common gastrocnemius tendon, equipped with a metal foil strain gauge (type
427 FLA-1, Tokyo Sokki Kenkyujo). We connected transducers to a micro-connector plug (15-way Micro-D, Farnell
428 Ltd, Leeds, UK) sutured to the bird’s dorsal synsacrum.

429 **Transducer recordings**

430 A lightweight shielded cable was used to connect the microconnector to data acquisition systems.
431 Sonomicrometry data were collected via a Sonometrics TRX analog data-acquisition device and PC interface
432 (TRX Series 8, Sonometrics, Ontario, Canada). Crystals were tested before surgery in a saline bath to confirm
433 distances measured by digital caliper matched those measured by the software. Occasional drop-outs and level-
434 shift artifacts in the sonomicrometry length signal (arising from variation in signal-to-noise characteristics) were
435 corrected within the Sonometrics software where possible and smoothed using cubic smoothing spline with a
436 tolerance of 0.1 in MATLAB (‘spaps’ function, Mathworks, Inc.; Natick, MA, USA). Tendon buckle signals
437 were fed through a bridge amplifier (Vishay 2120, Micro-Measurements, Raleigh, NC), and EMG signals were
438 amplified and bandpass filtered (10Hz and 3kHz) using GRASS pre-amplifiers (P511, Grass Instruments,
439 Warwick, RI). Signals were recorded at 10kHz using a 16-channel, 16-bit Biopac A/D acquisition device
440 (MP150, BIOPAC systems, Gotleta, CA, USA). Following experiments, birds were euthanized using an
441 intravenous injection of sodium pentobarbital (100 mg kg⁻¹) while under deep isoflurane anesthesia (4%, mask
442 delivery).

443

444 **Muscle morphology**

445 Postmortem, we recorded the morphology of the muscle and the location of transducers to confirm muscle
446 fascicle and tendon alignment. In the reinnervated cohort (this study) LG mass was 10.7 ± 2.7 g and total
447 gastrocnemius mass was 26.5 ± 5.1 g. In the intact cohort (Daley and Biewener 2011), LG mass was 10.2 ± 4.3 g,
448 and total gastrocnemius mass was 23.5 ± 7.4 g. These muscle masses are comparable to those measured from
449 intact guinea fowl in previous studies, representing approximately 0.5% body mass for LG and 1.3% body mass
450 for total gastrocnemius mass (Daley & Biewener 2003; Higham & Biewener 2008). This suggests full recovery
451 from denervation-induced muscle atrophy in the reinnervated cohort. Fascicle lengths for were 17 ± 1 mm and
452 18 ± 2 mm and pennation angles were $25 \pm 5^\circ$ and $24 \pm 5^\circ$ for reinnervated and intact LG, respectively. Crystal
453 alignment relative to the fascicle axis (α) was within 2° , indicating that errors due to misalignment were $< 0.1\%$.
454 We calibrated the tendon force buckle *in situ post mortem* by applying a series of known cyclical loads using a
455 force transducer (model 9203, Kistler, Amherst, MA), which yielded linear least-squares calibration slopes with
456 $R^2 > 0.97$.

457 **Level and obstacle terrain conditions**

458 We recorded trials on i) uniform level terrain and ii) terrain with repeating 5 cm obstacles, at the same treadmill
459 speeds, as in Daley and Biewener (2011). The treadmill belt (Woodway, Waukesha WI) was slatted black
460 rubber-coated steel with running surface 55.8 cm x 172.7 cm with clearance for obstacles beneath. Obstacles
461 were constructed from styrofoam reinforced with cardboard covered with black neoprene to form a light, stiff
462 surface. Waterproof glue (Shoe Goo, Eugene, OR, USA) secured heavy-duty fabric hook and loop fastener
463 (Velcro, Cheshire, UK) to the obstacle and treadmill surface. Four sequential slats of obstacles produced a
464 20 cm^2 continuous obstacle surface. Obstacles were encountered approximately every 4-5 strides, with some
465 variation due to varied stride length and treadmill station keeping. We recorded high-speed video at 250 Hz
466 (Photron, San Diego, CA, USA) for analysis of ankle kinematics, detection of stride timing and statistical coding
467 of strides in relation to obstacle encounters.

469 **Data Processing**

470 We assigned strides categories in relation to the obstacle encounters, using the approach in Daley and Biewener
471 (2011), based on the stride sequence of the instrumented leg: the stride prior to an obstacle contact (S -1),
472 obstacle contact strides (S 0), the stride following obstacle contact (S +1), and strides in flat terrain between
473 obstacles (S +2). All level terrain strides were assigned the same stride category (L). Note, this is simpler stride
474 coding than presented in Gordon and colleagues (2015), which also considered the timing of obstacle encounters
475 by the contralateral leg. Strides in which the contralateral limb had made contact with the obstacle in the

previous step are grouped here into the (S +2) category, for simplicity. This does not substantially alter the findings, because the coding was similar between intact and reinnervated birds, and the current analysis is focused on the shifts in LG force-length dynamics related to a direct obstacle encounter by the instrumented leg. Features of LG activation, force-length dynamics and work output were measured, similar to Daley and Biewener (2011). Raw EMG signals were used to calculate myoelectric intensity in time and frequency domains using wavelet decomposition (Daley et al. 2009; Gordon et al. 2015). This was used to calculate total myoelectric intensity per stride (E_{tot}) and mean frequency of muscle activation (E_{freq}). We calculated fractional fascicle length (L) from sonomicrometry data using mean length in level terrain as a reference length (L_o). Note, however, that L_o is not directly related to sarcomere length or optimal length for the isometric force-length curve, which were not measured. Fractional fascicle length was differentiated to obtain fascicle velocity (V, in lengths per second, Ls^{-1}). Shortening strains are negative. We multiplied fascicle velocity (in ms^{-1}) by tendon force (in Newtons, N) to calculate muscle power (Watts), which was integrated through time to calculate total work per stride (Joules, J; with shortening work being positive), and then normalized by muscle mass to obtain mass-specific muscle work (Jkg^{-1}). We also recorded muscle (fascicle) length, velocity and force at specified times to evaluate how strain and activation factors influence muscle force and work output. All data processing was completed using MATLAB (Mathworks, Inc.; Natick, MA, USA).

Statistics

The statistical analysis approach is similar to that used in Gordon and colleagues (2015) to investigate obstacle perturbation responses relative to steady state level terrain strides. We used a linear mixed-effects model ANOVA to test for significant effects of *treatment* (intact/reinnervated cohorts) and stride category (*stride ID*: Level, S -1, S 0, S +1, S +2) as fixed categorical factors, with individual (*ind*) included as a random effect. Statistical analysis was completed in Matlab using 'fitlme' and associated functions in the Statistics and Machine Learning Toolbox (Mathworks, Inc.; Natick, MA, USA). Several linear mixed effects models were evaluated:

- 1) 'Y ~ 1 + (1|ind)'
- 2) 'Y ~ 1 + treatment + (1|ind)'
- 3) 'Y ~ 1 + stride_ID + (1|ind)'
- 4) 'Y ~ 1 + stride_ID + treatment + (1|ind)'
- 5) 'Y ~ 1 + stride_ID *treatment + (1|ind)'

Model 5 (with the interaction term between fixed effects) was used as the final model, because it had the lowest AIC for 13 of 14 of the variables analyzed (AIC, Akaike, 1976). Model 4 had the lowest AIC for mean EMG frequency (E_{freq}), but the difference between Models 4 and 5 was not significant according to a likelihood ratio test. Therefore, for consistency, Model 5 was used for all variables. Posthoc pairwise comparisons were

509 calculated for the mean difference $\pm$ 95% confidence interval between intact and reinnervated treatment cohorts
510 and for the mean differences between level and obstacle strides categories within each treatment cohort. Pairwise
511 comparisons were calculated after removing the random effect of individual, because the intact and reinnervated
512 datasets came from different cohorts of individuals. We used False Discovery Rate to calculate an adjusted p-
513 value threshold to maintain a 5% false positive rate across all statistical tests, including fixed effects tests and
514 post-hoc pairwise comparisons (Benjamini, and Hochberg 1995).

515

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521

References

- Abelew, T. A., Miller, M. D., Cope, T. C. and Nichols, T. R. (2000). Local loss of proprioception results in disruption of interjoint coordination during locomotion in the cat. *Journal of Neurophysiology* 84, 2709-2714.
- Alvarez, F. J., Titus-Mitchell, H. E., Bullinger, K. L., Kraszpulski, M., Nardelli, P. and Cope, T. C. (2011). Permanent central synaptic disconnection of proprioceptors after nerve injury and regeneration. I. Loss of VGLUT1/IA synapses on motoneurons. *Journal of Neurophysiology* 106, 2450-2470.
- Askew, G. N. and Marsh, R. L. (1998). Optimal shortening velocity (V/V_{max}) of skeletal muscle during cyclical contractions: length-force effects and velocity-dependent activation and deactivation. *Journal of Experimental Biology* 201, 1527-1540.
- Askew, G. N. and Marsh, R. L. (2001). The mechanical power output of the pectoralis muscle of blue-breasted quail (*Coturnix chinensis*): the in vivo length cycle and its implications for muscle performance. *Journal of Experimental Biology* 204, 3587-3600.
- Azizi, E., Brainerd, E. L. and Roberts, T. J. (2008). Variable gearing in pennate muscles. *Proceedings of the National Academy of Sciences of the United States of America* 105, 1745-1750.
- Benjamini, Y. and Hochberg, Y. (1995) Controlling the false discovery rate: a practical and powerful approach to multiple testing. *Journal of the Royal Statistical Society: series B (Methodological)*, 57, 289-300.
- Birn-Jeffery, A.V., Hubicki, C.M., Blum, Y., Renjewski, D., Hurst, J., and Daley, M.A. (2014). Don't break a leg: Running birds from quail to ostrich prioritize leg safety and economy in uneven terrain. *Journal of Experimental Biology* 217, 3786-3796.
- Brandt, J., Evans, J.T., Mildenhall, T., Mulligan, A., Konieczny, A., Rose, S.J. and English, A.W. (2015) Delaying the onset of treadmill exercise following peripheral nerve injury has different effects on axon regeneration and motoneuron synaptic plasticity. *Journal of neurophysiology*, 113, 2390-2399.
- Boeltz, T., Ireland, M., Mathis, K., Nicolini, J., Poplavski, K., Rose, S.J., Wilson, E. and English, A.W. (2013) Effects of treadmill training on functional recovery following peripheral nerve injury in rats. *Journal of neurophysiology*, 109, 2645-2657.
- Brown, I. E., & Loeb, G. E. (2000). A reductionist approach to creating and using neuromusculoskeletal models. In *Biomechanics and Neural Control of Posture and Movement* (pp. 148-163). Springer New York.
- Bullinger K.L., Nardelli P., Pinter M.J., Alvarez F.J., Cope T.C. (2011). Permanent central synaptic disconnection of proprioceptors after nerve injury and regeneration. II. Loss of functional connectivity with motoneurons. *Journal of Neurophysiology* 106: 2471–2485.
- Carr, J. A., Chao, L. and Biewener, A.A. (2010). The effects of denervation and self-reinnervation in the guinea fowl lateral gastrocnemius *Online abstract: American Society of Biomechanics*, 26(24), 22.
- Chang, Y. H., Auyang, A. G., Scholz, J. P. and Nichols, T. R. (2009). Whole limb kinematics are preferentially conserved over individual joint kinematics after peripheral nerve injury. *Journal of Experimental Biology* 212, 3511-3521.
- Clark, A. J. and Higham, T. E. (2011). Slipping, sliding and stability: locomotor strategies for overcoming low-friction surfaces. *Journal of Experimental Biology*, 214, 1369-1378.
- Clemente, C. J., & Richards, C. (2012). Determining the influence of muscle operating length on muscle performance during frog swimming using a bio-robotic model. *Bioinspiration & Biomimetics*, 7(3), 036018.
- Cohen, A. H. (1992). The role of heterarchical control in the evolution of central pattern generators. *Brain, Behavior and Evolution*, 40(2–3), 112–124.
- Cope, T. C., Bonasera, S. J. and Nichols, T. R. (1994). Reinnervated muscles fail to produce stretch reflexes. *Journal of Neurophysiology*, 71, 817-820.
- Cox S.M., Easton K.L., Lear M.C., Marsh R.L., Delp S.L., Rubenson J. (2019). The interaction of compliance and activation on the force-length operating range and force generating capacity of skeletal muscle: a computational study using a guinea fowl musculoskeletal model. *Integrative Organismal Biology*, 2019 Sep 3;1(1):obz022.
- Daley, M. A. and Biewener, A. A. (2003). Muscle force-length dynamics during level versus incline locomotion: a comparison of in vivo performance of two guinea fowl ankle extensors. *Journal of Experimental Biology*. 206, 2941-2958.
- Daley, M. A. and Biewener, A. A. (2011). Leg muscles that mediate stability: mechanics and control of two distal extensor muscles during obstacle negotiation in the guinea fowl. *Philosophical transactions of the Royal Society of London. Series B, Biological sciences*, 366, 1580-1591.
- Daley, M. A., & Birn-Jeffery, A. (2018). Scaling of avian bipedal locomotion reveals independent effects of body mass and leg posture on gait. *Journal of Experimental Biology*, 221(10), jeb152538.
- Daley, Monica A; Gordon, Joanne C; Biewener, Andrew A (2020), Dataset for 'Tuning of feedforward control enables stable muscle force-length dynamics after loss of autogenic proprioceptive feedback', v6, UC Irvine, Dataset, <https://doi.org/10.7280/D11H49>
- Daley, M. A., Voloshina, A., & Biewener, A. A. (2009). The role of intrinsic muscle mechanics in the neuromuscular control of stable running in the guinea fowl. *The Journal of Physiology*, 587(11), 2693-2707.

- Deban, S. M. and Carrier, D. R. (2002). Hypaxial muscle activity during running and breathing in dogs. *Journal of Experimental Biology*, 205, 1953-1967.
- Dick, T. J., Punith, L. K., & Sawicki, G. S. (2019). Humans falling in holes: Adaptations in lower-limb joint mechanics in response to a rapid change in substrate height during human hopping. *Journal of the Royal Society Interface*, 16(159), 20190292.
- Donelan, J. M. and Pearson, K. G. (2004). Contribution of force feedback to ankle extensor activity in decerebrate walking cats. *Journal of Neurophysiology*, 92, 2093-2104.
- Dorward, P. K. (1970). Response characteristics of muscle afferents in the domestic duck. *Journal of Physiology*, 211(1), 1-17.
- Drew, T. and Marigold, D.S. (2015) Taking the next step: cortical contributions to the control of locomotion. *Current opinion in neurobiology*, 33:25-33
- Edman, K. A. P. (1975). Mechanical deactivation induced by active shortening in isolated muscle fibres of the frog. *Journal of Physiology*, 246, 255-275
- Edman, K. A., Elzinga, G., & Noble, M. I. (1978). Enhancement of mechanical performance by stretch during tetanic contractions of vertebrate skeletal muscle fibres. *Journal of Physiology*, 281(1), 139-155.
- Edman, K. (1980). Depression of mechanical performance by active shortening during twitch and tetanus of vertebrate muscle fibres. *Acta Physiologica Scandinavica*, 109(1), 15-26.
- Edman, K. A. P. (2012). Residual force enhancement after stretch in striated muscle. A consequence of increased myofilament overlap? *Journal of Physiology*, 590(6), 1339-1345.
- Ferris, D. P., Liang, K., & Farley, C. T. (1999). Runners adjust leg stiffness for their first step on a new running surface. *Journal of Biomechanics*, 32(8), 787-794.
- Frigon, A. and Rossignol, S. (2006). Experiments and models of sensorimotor interactions during locomotion. *Biological Cybernetics* 95, 607-627.
- Gatesy, S. M., & Biewener, A. A. (1991). Bipedal Locomotion—Effects of Speed, Size and Limb Posture in Birds and Humans. *Journal of Zoology*, 224, 127-147.
- Gordon, J. C., Rankin, J. W., & Daley, M. A. (2015). How do treadmill speed and terrain visibility influence neuromuscular control of guinea fowl locomotion? *Journal of Experimental Biology*, 218(19), 3010-3022. doi:10.1242/jeb.104646
- Gordon, T. and Stein, R. B. (1982). Time course and extent of recovery in reinnervated motor units of cat triceps surae muscles. *Journal of Physiology*, 323, 307-323.
- Grillner, S. (2011). Control of Locomotion in Bipeds, Tetrapods, and Fish. *Comprehensive Physiology 2011, Supplement 2: Handbook of Physiology, The Nervous System, Motor Control*: (1179-1236).
- Haiden, G.J. and Awad, E.A., (1981). The ultrastructure of the avian Golgi tendon organ. *The Anatomical Record*, 200(2), 153-161.
- Heglund, N. C., Cavagna, G. A., & Taylor, C. R. (1982). Energetics and mechanics of terrestrial locomotion. III. Energy changes of the centre of mass as a function of speed and body size in birds and mammals. *Journal of Experimental Biology*, 97(1), 41-56.
- Herzog, W. (2004). History dependence of skeletal muscle force production: Implications for movement control. *Human Movement Science*, 23(5), 591-604.
- Herzog, W. (2014). Mechanisms of enhanced force production in lengthening (eccentric) muscle contractions. *Journal of Applied Physiology*, 116(11), 1407-1417.
- Ijspeert, A. J. (2014). Biorobotics: Using robots to emulate and investigate agile locomotion. *Science*, 346(6206), 196-203.
- Josephson, R. K. (1999). Dissecting muscle power output. *Journal of Experimental Biology* 202, 3369-3375.
- Kuo, A. D. (2002). The relative roles of feedforward and feedback in the control of rhythmic movements. *Motor control* 6, 129-145.
- Lam, T., and Pearson K.G. (2002) The role of proprioceptive feedback in the regulation and adaptation of locomotor activity. In *Sensorimotor Control of Movement and Posture* (pp. 343-355). Springer, Boston, MA.
- Loeb, G. E., Brown, I. E., & Cheng, E. J. (1999). A hierarchical foundation for models of sensorimotor control. *Experimental Brain Research*, 126(1), 1-18.
- Lyle M.A., Prilutsky B.I., Gregor R.J., Abelew T.A., Nichols T.R. (2016) Self-reinnervated muscles lose autogenic length feedback, but intermuscular feedback can recover functional connectivity. *Journal of Neurophysiology*. 116(3):1055-67.
- Lyle, M. A., & Nichols, T. R. (2018). Patterns of intermuscular inhibitory force feedback across cat hindlimbs suggest a flexible system for regulating whole limb mechanics. *Journal of Neurophysiology*, 119(2), 668-678.
- Maas, H., Prilutsky, B. I., Nichols, T. R. and Gregor, R. J. (2007). The effects of self-reinnervation of cat medial and lateral gastrocnemius muscles on hindlimb kinematics in slope walking. *Experimental Brain Research* 181, 377-393.
- Maier, A. (1992). The avian muscle spindle. *Anatomy and Embryology*, 186(1), 1-25.
- Marigold, D.S. and Drew, T. (2017) Posterior parietal cortex estimates the relationship between object and body location during locomotion. *eLife*, 6, p.e28143.

638 More, H. L., & Donelan, J. M. (2018). Scaling of sensorimotor delays in terrestrial mammals. *Proceedings of the Royal*
639 *Society B: Biological Sciences*, 285(1885), 20180613.

640 More, H. L., Hutchinson, J. R., Collins, D. F., Weber, D. J., Aung, S. K., & Donelan, J. M. (2010). Scaling of sensorimotor
641 control in terrestrial mammals. *Proceedings of the Royal Society B: Biological Sciences*, 277(1700), 3563–3568.

642 Moritz, C. T., & Farley, C. T. (2004). Passive dynamics change leg mechanics for an unexpected surface during human
643 hopping. *Journal of Applied Physiology*, 97(4), 1313–1322. <https://doi.org/10.1152/jappphysiol.00393.2004>

644 Nelson, F. E., Gabaldón, A. M., & Roberts, T. J. (2004). Force–velocity properties of two avian hindlimb muscles.
645 *Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology*, 137(4), 711–721.

646 Nichols, T.R. (1989) The organization of heterogenic reflexes among muscles crossing the ankle joint in the decerebrate cat.
647 *The Journal of Physiology*, 410(1), 463–477.

648 Nishikawa, K., Biewener, A. A., Aerts, P., Ahn, A. N., Chiel, H. J., Daley, M. A., Daniel, T. L., Full, R. J., Hale, M. E.,
649 Hedrick, T. L. et al. (2007). Neuromechanics: an integrative approach for understanding motor control. *Integrative*
650 *and Comparative Biology* 47, 16–54.

651 Nishikawa, K. C., Monroy, J. A., Uyeno, T. E., Yeo, S. H., Pai, D. K., & Lindstedt, S. L. (2012). Is titin a ‘winding
652 filament’? A new twist on muscle contraction. *Proceedings of the Royal Society of London B: Biological Sciences*,
653 279, 981–990.

654 Nishikawa, K. C., Monroy, J. A., & Tahir, U. (2018). Muscle function from organisms to molecules. *Integrative and*
655 *Comparative Biology*, 58(2), 194–206.

656 Pearson, K. (2000). Motor systems. *Current Opinion in Neurobiology*, 10(5), 649–654.

657 Pearson, K., & Gramlich, R. (2010). Updating neural representations of objects during walking. *Annals of the New York*
658 *Academy of Sciences*, 1198(1), 1–9.

659 Pearson, K. G., Misiaszek, J. E. and Hulliger, M. (2003). Chemical ablation of sensory afferents in the walking system of
660 the cat abolishes the capacity for functional recovery after peripheral nerve lesions. *Experimental Brain Research*
661 150, 50–60.

662 Poore, S. O., Ashcroft, A., Sanchez-Haiman, A., & Goslow, G. (1997). The contractile properties of the M.
663 supracoracoideus in the pigeon and starling: A case for long-axis rotation of the humerus. *Journal of Experimental*
664 *Biology*, 200(23), 2987–3002.

665 Potocanac, Z., de Bruin, J., van der Veen, S., Verschueren, S., van Dieën, J., Duysens, J. and Pijnappels, M. (2014) Fast
666 online corrections of tripping responses. *Experimental brain research*, 232(11): 3579–3590.

667 Prochazka, A. and Ellaway, P. (2012). Sensory systems in the control of movement. *Comprehensive Physiology* 2, 2615–
668 2627.

669 Proske, U., & Gandevia, S. C. (2012). The proprioceptive senses: their roles in signaling body shape, body position and
670 movement, and muscle force. *Physiological Reviews*, 92(4), 1651–1697.

671 Richards, C. T. (2011). Building a robotic link between muscle dynamics and hydrodynamics. *Journal of Experimental*
672 *Biology*, 214(14), 2381–2389.

673 Roberts, T. J., Marsh, R. L., Weyand, P. G. and Taylor, C. R. (1997). Muscular force in running turkeys: the economy of
674 minimizing work. *Science* 275, 1113–1115.

675 Robertson, B. D., & Sawicki, G. S. (2015). Unconstrained muscle-tendon workloops indicate resonance tuning as a
676 mechanism for elastic limb behavior during terrestrial locomotion. *Proceedings of the National Academy of*
677 *Sciences*, 112(43), E5891–E5898.

678 Rode, C., Siebert, T., & Blickhan, R. (2009). Titin-induced force enhancement and force depression: A ‘sticky-spring’
679 mechanism in muscle contractions? *Journal of Theoretical Biology*, 259(2), 350–360.

680 Ross, K. T. and Nichols, T. R. (2009). Heterogenic feedback between hindlimb extensors in the spontaneously locomoting
681 premammillary cat. *Journal of Neurophysiology* 101, 184–197.

682 Rossignol, S., Dubuc, R. and Gossard, J. P. (2006). Dynamic sensorimotor interactions in locomotion. *Physiological*
683 *Reviews* 86, 89–154.

684 Roth, E., Sponberg, S., & Cowan, N. (2014). A comparative approach to closed-loop computation. *Current Opinion in*
685 *Neurobiology*, 25, 54–62.

686 Safavynia, S.A. and Ting, L.H. (2013) Long-latency muscle activity reflects continuous, delayed sensorimotor feedback of
687 task-level and not joint-level error. *Journal of neurophysiology*, 110(6), 1278–1290.

688 Sawicki, G.S., Robertson, B.D., Azizi, E. and Roberts, T.J., (2015). Timing matters: tuning the mechanics of a muscle–
689 tendon unit by adjusting stimulation phase during cyclic contractions. *Journal of Experimental Biology*, 218(19),
690 3150–3159.

691 Sharp, A. A., & Bekoff, A. (2015). Pyridoxine treatment alters embryonic motility in chicks: Implications for the role of
692 proprioception. *Developmental Psychobiology*, 57(2), 271–277.

693 Sherrington, C. S., & Laslett, E. E. (1903). Observations on some spinal reflexes and the interconnection of spinal segments.
694 *Journal of Physiology*, 29(1), 58–96.

695 Sherrington, C. S. (1910). Remarks on the reflex mechanism of the step. *Brain* 33.1 1–25.

696 Spotnitz, W. D. (2010). Fibrin sealant: past, present, and future: a brief review. *World Journal of Surgery* 34, 632–634.

697 Taylor, C. R., Heglund, N. C., & Maloiy, G. (1982). Energetics and mechanics of terrestrial locomotion. I. Metabolic energy
698 consumption as a function of speed and body size in birds and mammals. *Journal of Experimental Biology*, 97(1),
699 1–21.

700 Todorov, E. (2004). Optimality principles in sensorimotor control. *Nature Neuroscience*, 7(9), 907-915.

701 Vannucci, B., Santosa, K. B., Keane, A. M., Jablonka-Shariff, A., Lu, C., Yan, Y., ... Snyder-Warwick, A. K. (2019). What
702 is Normal? Neuromuscular junction reinnervation after nerve injury. *Muscle & Nerve*.60:604–612

703 Wagner, M. J., & Smith, M. A. (2008). Shared internal models for feedforward and feedback control. *Journal of*
704 *Neuroscience*, 28(42), 10663-10673.

705 Wolpert, D. M., Diedrichsen, J., & Flanagan, J. R. (2011). Principles of sensorimotor learning. *Nature Reviews*
706 *Neuroscience*, 12(12), 739-751.

707 Yakovenko, S., Gritsenko, V. and Prochazka, A. (2004). Contribution of stretch reflexes to locomotor control: a modeling
708 study. *Biological Cybernetics* 90, 146-155.

709 Yeo, S. H., Monroy, J. A., Lappin, A. K., Nishikawa, K. C., & Pai, D. K. (2013). Phenomenological models of the dynamics
710 of muscle during isotonic shortening. *Journal of Biomechanics*, 46(14), 2419-2425.

711

Figure legends and Tables

Figure 1. Protocol for bilateral self-reinnervation of the lateral gastrocnemius (LG), followed by transducer implantation for *in vivo* recordings of muscle force (tendon buckle), fascicle length (sonomicrometry crystals) and electromyographic activity (EMG).

Figure 2. Muscle trajectories (mean $\pm$ 95% ci) during obstacle negotiation for intact and reinnervated lateral gastrocnemius (iLG: blue, top, rLG: orange, bottom), averaged over the stride cycle from mid-swing to mid-swing. Averages are shown for a 4-stride sequence in obstacle terrain, with steady level terrain means as a reference, in grey. The shaded box is an obstacle encounter (S 0). Obstacle terrain strides are coded as in Daley and Biewener 2011, for strides preceding (S -1), on (S 0) and following obstacle contact (S +1), with S +2 including all other strides between obstacles. Trajectories are fractional muscle fascicle length (top), muscle-tendon force (middle) and rectified myoelectric activity (EMG). Triangles indicate the timing of foot-ground contact (grey: level terrain, black: obstacle terrain). Example data is shown from one individual in each treatment cohort. See **Figure 2- figure supplement 1** for details on stride-cycle cutting and categorization in an example stride sequence in obstacle terrain.

Figure 2-figure supplement 1. Example 6-stride sequence of *in vivo* muscle recordings of the reinnervated lateral gastrocnemius (rLG) in the right leg, running at 1.7ms^{-1} on the obstacle treadmill. Muscle length (top, orange), force (bottom, black) and activation (rectified EMG, bottom, orange) are shown, with triangles indicating the time of foot-ground contact, a shaded box indicating an obstacle encounter stride (S 0), and vertical lines indicating the mid-swing cut points between stride cycles. Stride categories were identified from video. Grey silhouettes at the top illustrate the leg posture at the time of foot contact. Strides cycles were cut based on a minimum in muscle-tendon force after it was low-pass filtered with a 6th order Butterworth filter with a cutoff frequency of 3.4Hz. This resulted in a sinusoidal trajectory with a mid-swing minimum, which was confirmed against video to correspond to when the swing leg crossed vertical. Note that between the last two strides, the contralateral leg stepped on the obstacle, leading to a downward step of the instrumented leg in the final stride. For simplicity, these strides are group with the ‘mid-flat’ strides S+2 (as in Daley and Biewener 2011) because the focus of the current analysis is the direct response to the obstacle encounter (S 0).

Figure 3. Force-length work loops for iLG (top) and rLG (bottom), for a single individual from each treatment cohort (intact/reinnervated, mean $\pm$ 95% ci). Level mean in grey and obstacle strides in colored lines (iLG: blue, rLG: orange). Stride categories as in Figure 2, where the shaded box is an obstacle encounter (S 0). Triangles indicate the timing of foot-ground contact and arrows indicate the direction of the work loop, with a counter-clockwise loop corresponding to net positive muscle work.

Figure 4. A) Distributions of muscle total work output (W_{net}), peak force (F_{pk}), velocity and length at peak force (V_{pkF} , L_{pkF}) across stride categories for iLG (blue) and rLG (orange). Circles indicate group means. Lines connect means between stride categories, to highlight the shifts in relation to obstacle encounters (S 0). **B)** Pairwise mean differences (mean $\pm$ 95% ci) for fixed effect categories, between intact and reinnervated treatment cohorts (grey bar), and between obstacle stride categories compared to level means, within treatment cohorts (colored bars). See Tables 1& 2 for full statistics results and summary data.

Figure 5. A) Distributions of total intensity EMG activity (E_{tot}), duration of activity (E_{dur}) and mean frequency of activity (E_{freq}) across stride categories for iLG (blue) and rLG (orange). Circles indicate group means. Lines connect means between stride categories, to highlight the shifts in relation to obstacle encounters (S 0). **B)** Pairwise mean differences (mean $\pm$ 95% ci) for fixed effects, as presented as in Figure 4. See Tables 1& 2 for full statistics results and summary data.

Figure 5-figure supplement 1. Distribution of EMG activation frequency (mean $\pm$ 95% ci) for intact LG (top, blue) and reinnervated LG (bottom, orange), during level running (dark grey lines) and obstacle encounters (S 0, colored lines). Note the shift in peak frequency of EMG activity in the reinnervated LG for both level terrain and obstacle strides, suggesting recruitment of faster motor units.

Figure. 6. Phase relationship (E_{phase}) between length and EMG activation **A)** Average steady-state length and activation trajectories for iLG and rLG in level terrain, aligned in time based on peak length during the swing phase, before foot-substrate contact. Black dot and vertical dashed line indicate the time of peak fascicle length. Triangles indicate timing of foot contact. **B)** Pairwise mean differences in E_{phase} (mean $\pm$ 95% ci) between intact and reinnervated treatment cohorts (grey), and obstacle stride categories compared to level terrain within each cohort (colored bars). E_{phase} is reported in the ANOVA tables as a fraction of the stride cycle but is reported in milliseconds here.

Figure. 7. Deviations from steady state in the stride cycle trajectories of muscle length, force and activation, between obstacle strides (S 0) and level strides (grand mean $\pm 95\%$ ci across individuals). The horizontal zero line indicates no difference from steady state in S 0. The stride cycle is from mid-swing to mid-swing, as in Figure 2. A black asterisk (*) indicates the first timepoint in each trajectory that differs significantly from the level mean. The dashed vertical line and arrow indicating ‘anticipatory increase’ highlights a significant increase in EMG that starts *before* deviations length and force in S 0. In **A**) (iLG), solid vertical lines and yellow fill indicates a 2nd period of significantly increased EMG in late stance that correlates with increased fascicle length and force, suggesting a reflex response. In **B**) (rLG), the anticipatory increase in EMG is present; however, wide confidence intervals for EMG in late stance indicates inconsistent patterns of activity across individuals, despite similar increases in length and force as iLG. This suggests disrupted autogenic feedback and idiosyncratic heterogenic feedback patterns across individuals (**Figure. 7-figure supplement 1**).

Figure. 7-figure supplement 1. Average stride cycle trajectories (mean $\pm 95\%$ ci) for fascicle length, muscle-tendon force and myoelectric activity (EMG) during obstacle encounter strides (S 0, orange) compared to the level terrain averaged (grey), for all birds with reinnervated LG. Although the deviations in length and force during obstacle encounters is qualitatively similar across individuals, the shifts in EMG activation in the latter half of stance varies substantially across individuals, with some individuals showing reflex inhibition of activity (Ind 13, Ind 25) and others showing reflex excitation.

Figure. 8. Ankle kinematics in guinea fowl with intact and reinnervated lateral gastrocnemius (LG). **A**) Example ankle joint angle trajectories for a bird with intact LG (blue, top) and a bird with reinnervated LG (orange, below), running in obstacle terrain (solid lines) with level terrain (grey dashed lines). **B**) Pairwise mean differences (mean $\pm 95\%$ ci) between intact and reinnervated treatment cohorts (grey), and obstacle stride categories compared to level terrain within each treatment cohort (intact: blue, reinnervated: orange). In obstacle strides (S 0, shaded box), the ankle is more flexed at foot contact in reinnervated compared to intact birds. Reinnervated birds show a shorter stride period in S -1, preceding the obstacle encounter, suggesting increased anticipatory preparation. (See **Figure. 8- source data 1** for statistical results on ankle angle at the time of foot contact).

Figure. 8- source data 1. ANOVA results for ankle angle at time of foot contact. F-statistics, p-values and posthoc pairwise comparisons for linear mixed effect model ANOVA with fixed effects of treatment cohort (*treatment*: intact, reinnervated) and stride category (*stride ID*) and the interaction *treatment* x *stride ID*. Posthoc pairwise mean differences (mean $\pm 95\%$ ci) are shown between intact and reinnervated treatment cohorts (left), and between obstacle stride categories compared to level stride means, within treatment cohorts (intact/reinnervated).

Figure 9. Schematic illustration of neuromechanical control system, indicating hypothesized mechanisms for the observed changes in self-reinnervated lateral gastrocnemius of guinea fowl. Green text indicates the *in vivo* experimental measures used to infer sensorimotor control mechanisms. Differences in muscle dynamics between intact and reinnervated cohorts suggest that guinea fowl use a combination of feedforward and intrinsic mechanical mechanisms to compensate for disrupted proprioceptive reflexes, suggesting interconnected plasticity of neural and musculoskeletal mechanisms in the recovery from nerve injury.

797

798 **Table 1.** F-statistics for linear mixed effect model ANOVA with fixed effects of treatment cohort (*treatment*: intact,
799 reinnervated) and stride category (*stride ID*) and the interaction *treatment* x *stride ID* on measures of muscle contraction
800 mechanics and activation. Bolding indicates statistical significance using FDR corrected threshold ($p \leq 0.0263$, see
801 Methods). Degrees of freedom for fixed effects were *treatment* = 1, *stride ID* = 4, *interaction* = 4, and error = 2529. See
802 **Table 1 - source data 1** for p-values.

Variable	F-statistic		
	<i>treatment</i>	<i>stride ID</i> :	<i>interaction</i>
W_{net}	2.44	172.04	7.44
F_{pk}	0.52	30.12	61.73
L_{pkF}	1.52	238.03	39.52
V_{pkF}	11.43	28.09	8.73
T_{force}	0.27	99.09	18.32
T_{stride}	0.10	12.17	17.82
E_{tot}	0.01	49.05	3.51
E_{freq}	3.93	8.71	1.85
E_{phase}	5.72	2.34	7.64
E_{dur}	10.02	8.86	10.69

803

804 **Table 1 - source data 1.** P-values linear mixed effect model ANOVA with fixed effects of treatment cohort (*treatment*:
805 intact, reinnervated) and stride category (*stride ID*) and the interaction *treatment* x *stride ID*.

806 **Table 2. Pairwise mean differences** (mean $\pm$ 95% ci) between intact and reinnervated treatment cohorts (left),
807 and between obstacle stride categories compared to level stride means, within treatment cohorts
808 (intact/reinnervated). Bolding indicates statistical significance using FDR corrected threshold ($p \leq 0.0263$). See
809 Table 2- source data 1 for p-values.

810

Variable	Treatment cohort	Intact				Reinnervated			
		S -1	S 0	Str +1	S +2	S -1	S 0	Str +1	S +2
W_{net}	-0.47 $\pm$ 0.59	-0.91$\pm$0.60	3.60$\pm$0.57	0.00 $\pm$ 0.59	-0.86$\pm$0.34	0.34 $\pm$ 0.55	3.88$\pm$0.60	0.49 $\pm$ 0.70	-0.19 $\pm$ 0.46
F_{pk}	-0.02 $\pm$ 0.06	-0.04 $\pm$ 0.07	0.17$\pm$0.06	0.04 $\pm$ 0.07	-0.04 $\pm$ 0.04	0.09$\pm$0.06	0.62$\pm$0.07	0.10$\pm$0.08	0.05 $\pm$ 0.05
L_{pkF}	-0.02 $\pm$ 0.04	-0.02$\pm$0.01	0.10$\pm$0.01	-0.01 $\pm$ 0.01	-0.02$\pm$0.01	-0.05$\pm$0.01	0.14$\pm$0.01	-0.06$\pm$0.02	-0.04$\pm$0.01
V_{pkF}	-1.89$\pm$1.10	0.07 $\pm$ 0.45	-1.26$\pm$0.43	0.00 $\pm$ 0.45	0.11 $\pm$ 0.26	0.60$\pm$0.42	-0.39 $\pm$ 0.46	0.63$\pm$0.53	0.81$\pm$0.35
T_{force}	0.02 $\pm$ 0.07	-0.01 $\pm$ 0.02	0.11$\pm$0.02	-0.01 $\pm$ 0.02	-0.01 $\pm$ 0.01	-0.07$\pm$0.02	0.07$\pm$0.02	-0.06$\pm$0.02	-0.06$\pm$0.02
T_{stride}	0.00 $\pm$ 0.02	-0.01 $\pm$ 0.02	0.03$\pm$0.02	-0.02$\pm$0.02	0.01 $\pm$ 0.01	-0.07$\pm$0.02	0.04$\pm$0.02	-0.04$\pm$0.03	-0.03$\pm$0.02
E_{tot}	0.01 $\pm$ 0.22	0.12 $\pm$ 0.18	0.76$\pm$0.18	0.11 $\pm$ 0.18	0.12$\pm$0.11	0.16 $\pm$ 0.17	0.74$\pm$0.19	0.39$\pm$0.22	0.09 $\pm$ 0.14
E_{freq}	55.70 $\pm$ 55.12	3.05 $\pm$ 9.44	-15.05$\pm$9.04	-1.46 $\pm$ 9.34	-0.46 $\pm$ 5.43	-2.86 $\pm$ 8.75	-22.06$\pm$9.52	-6.81 $\pm$ 11.04	-7.68$\pm$7.28
E_{phase}	-0.06$\pm$0.05	-0.01 $\pm$ 0.02	0.00 $\pm$ 0.02	0.00 $\pm$ 0.02	-0.01 $\pm$ 0.01	0.01 $\pm$ 0.02	0.04$\pm$0.02	-0.01 $\pm$ 0.02	0.00 $\pm$ 0.01
E_{dur}	0.08$\pm$0.05	0.00 $\pm$ 0.03	0.04$\pm$0.02	-0.01 $\pm$ 0.02	0.00 $\pm$ 0.01	-0.04$\pm$0.02	-0.01 $\pm$ 0.03	0.00 $\pm$ 0.03	-0.03$\pm$0.02

811

812 **Table 2- source data 1.** P-values for posthoc pairwise mean differences between intact and reinnervated
813 treatment cohorts (left column) and between obstacle stride categories compared to the level terrain means,
814 within treatment cohorts (intact/reinnervated).

Figure 1

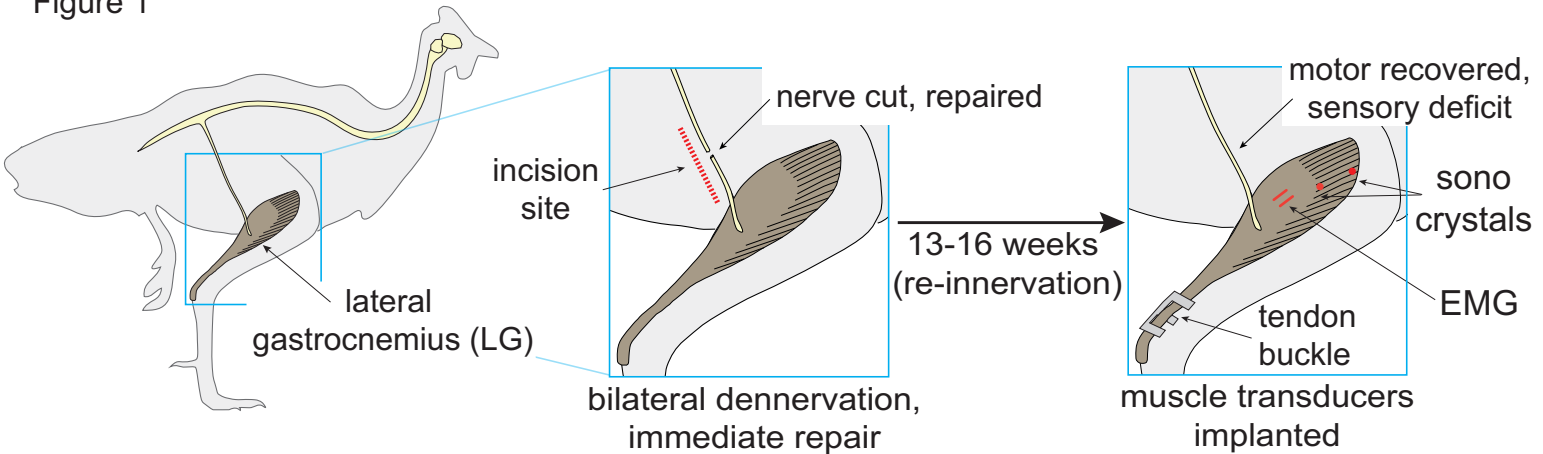


Figure 2

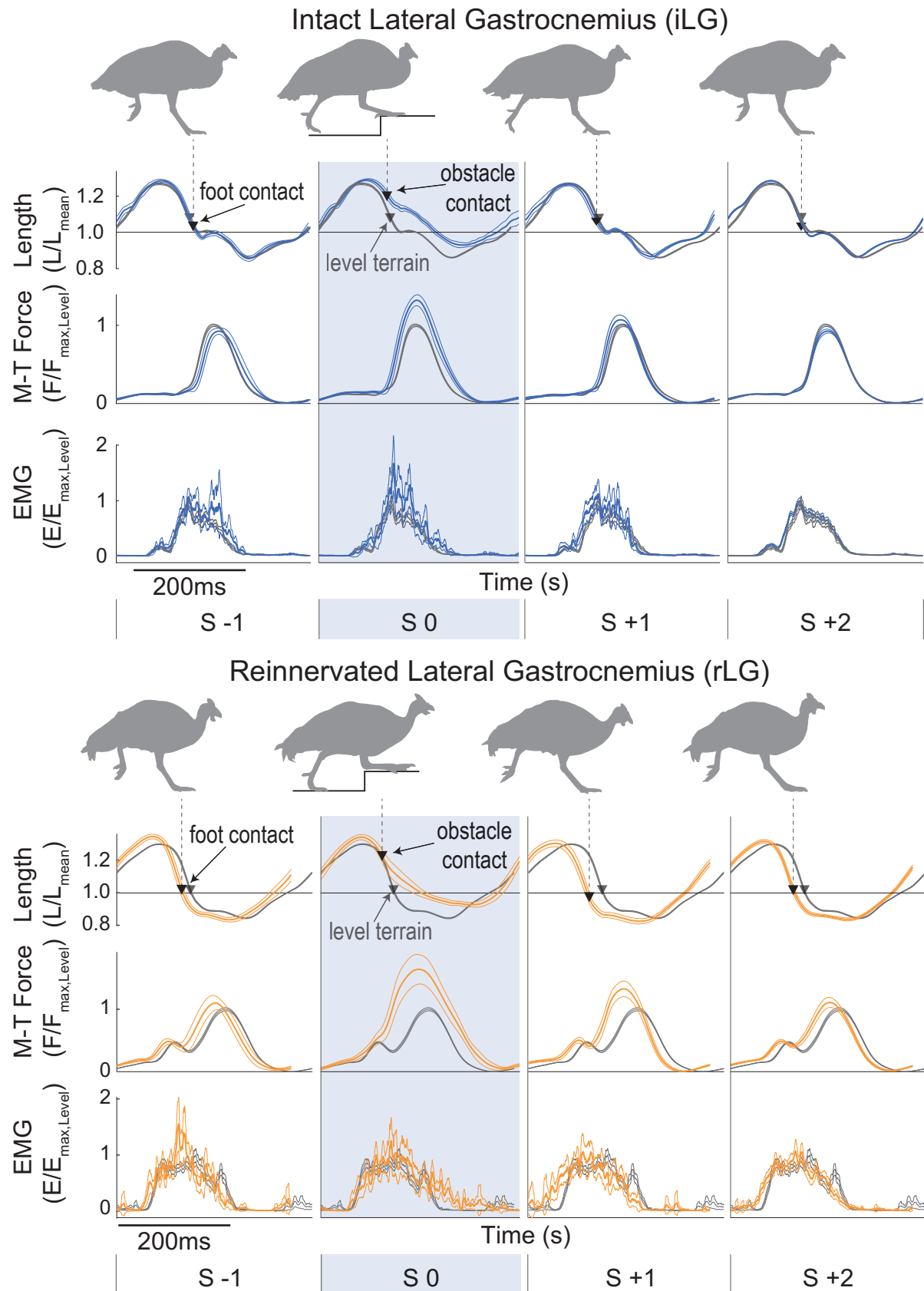


Figure 2-figure supplement 1

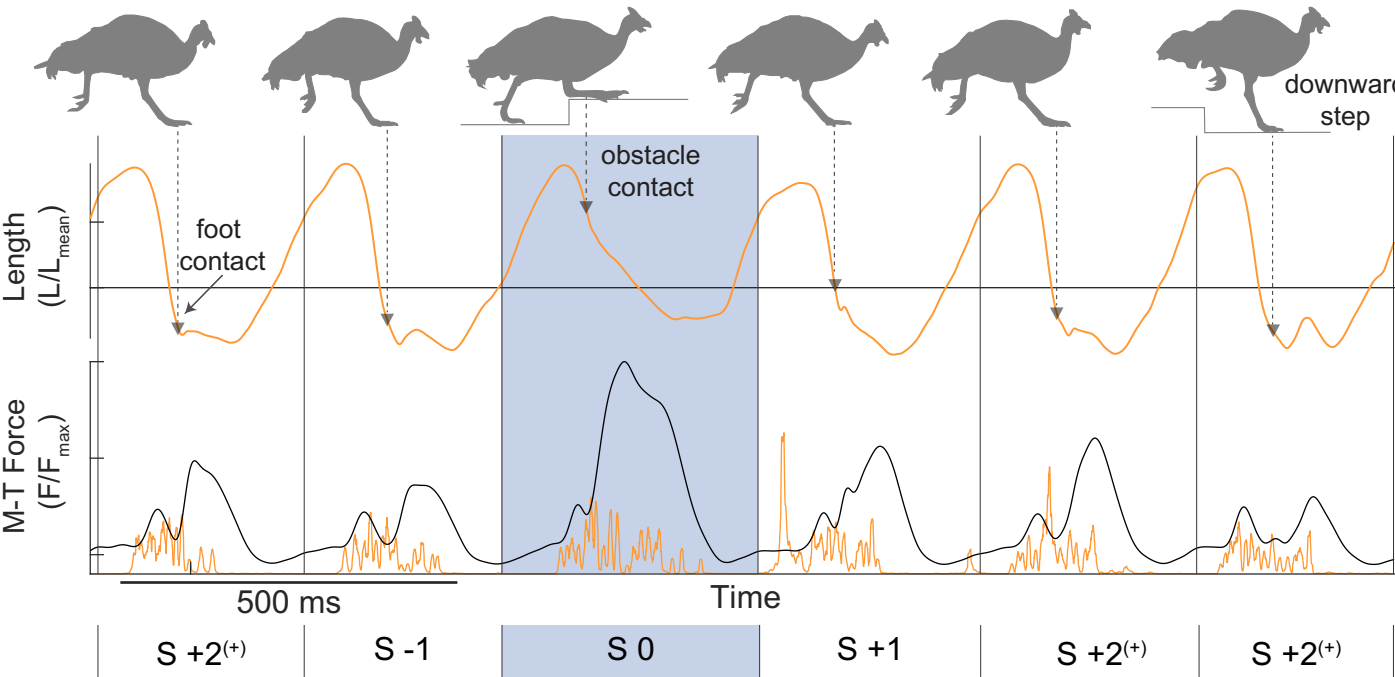


Figure 3

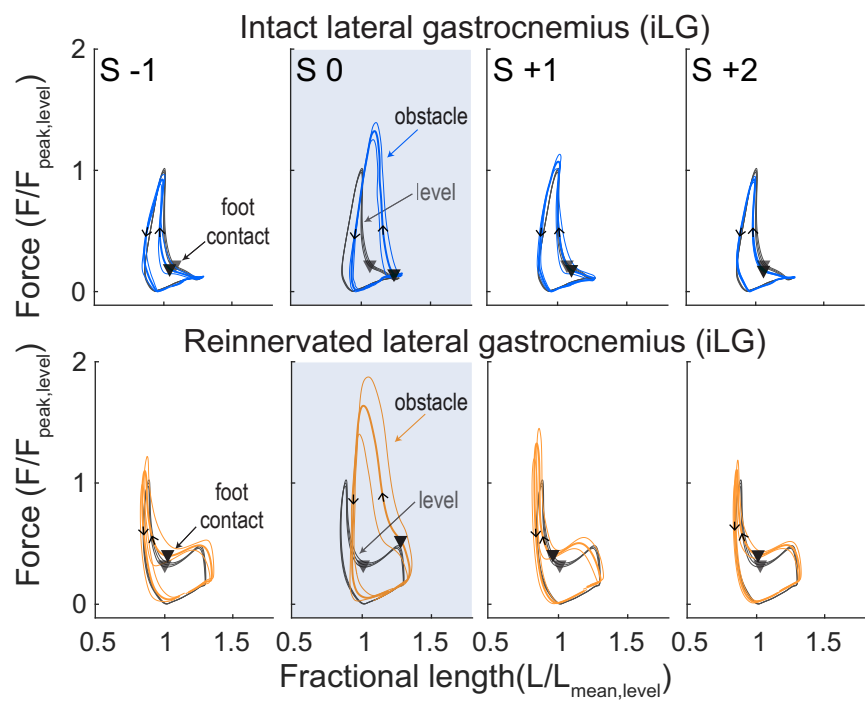


Figure 4

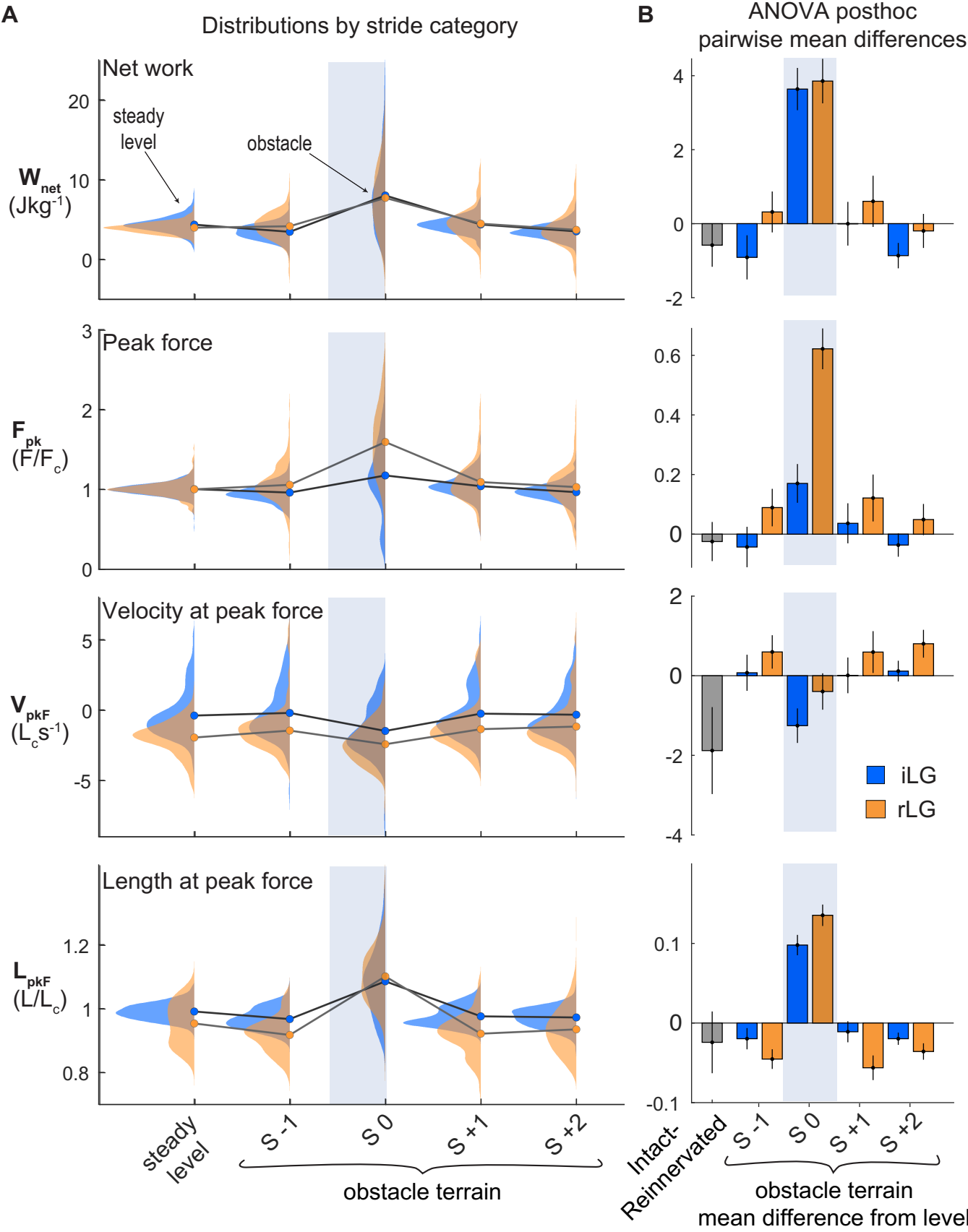


Figure 5

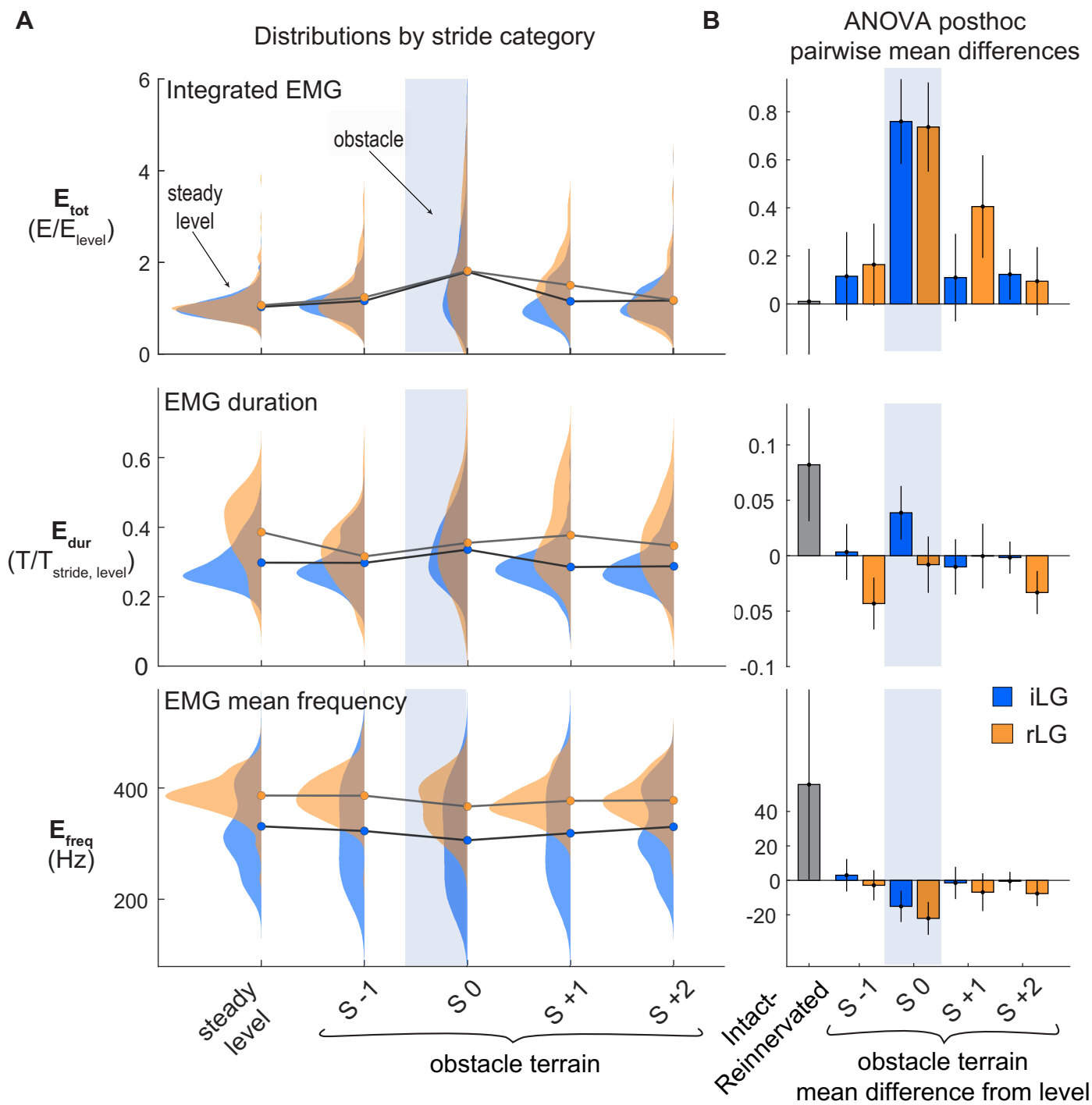


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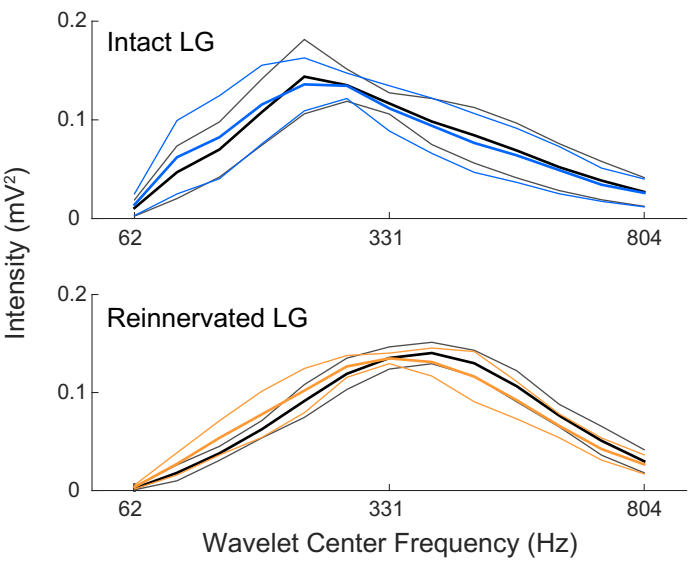


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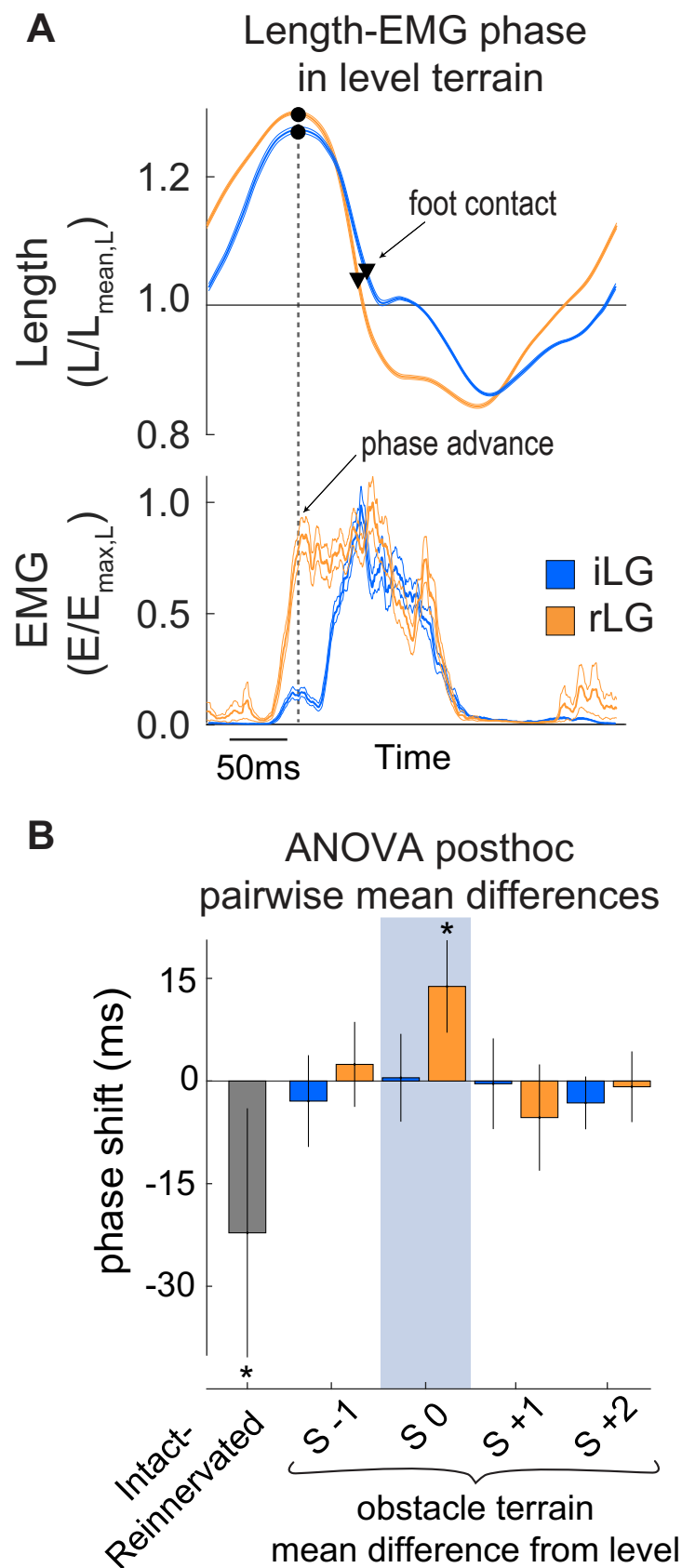


Figure 7

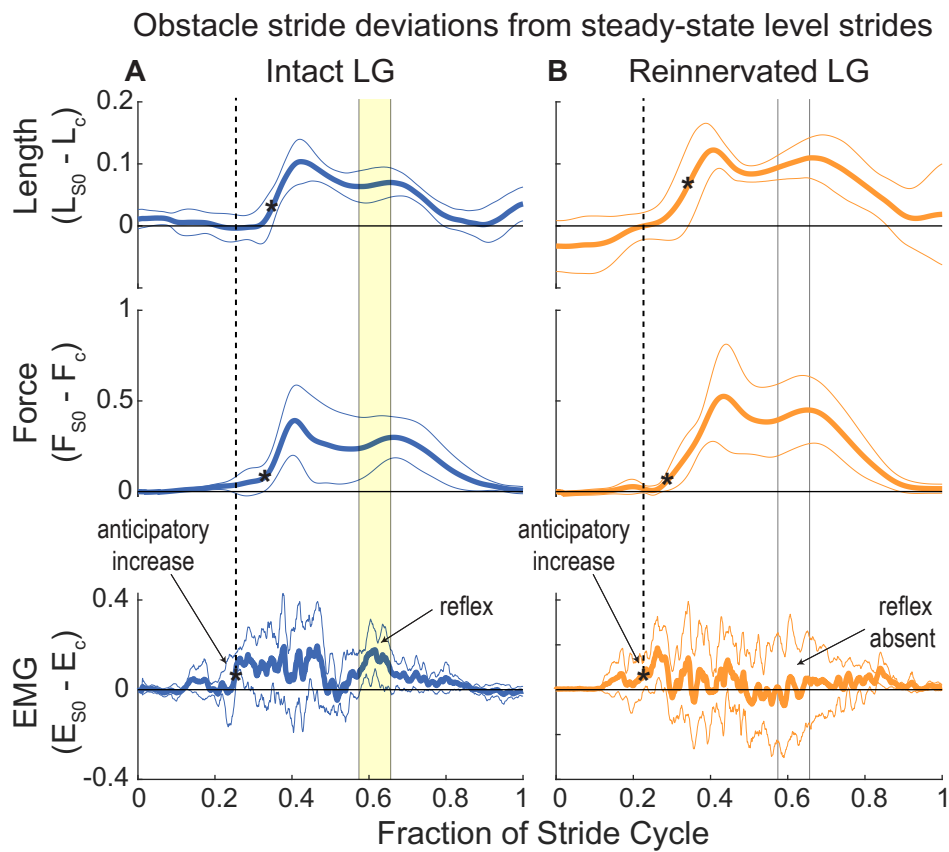


Figure 7-figure supplement 1

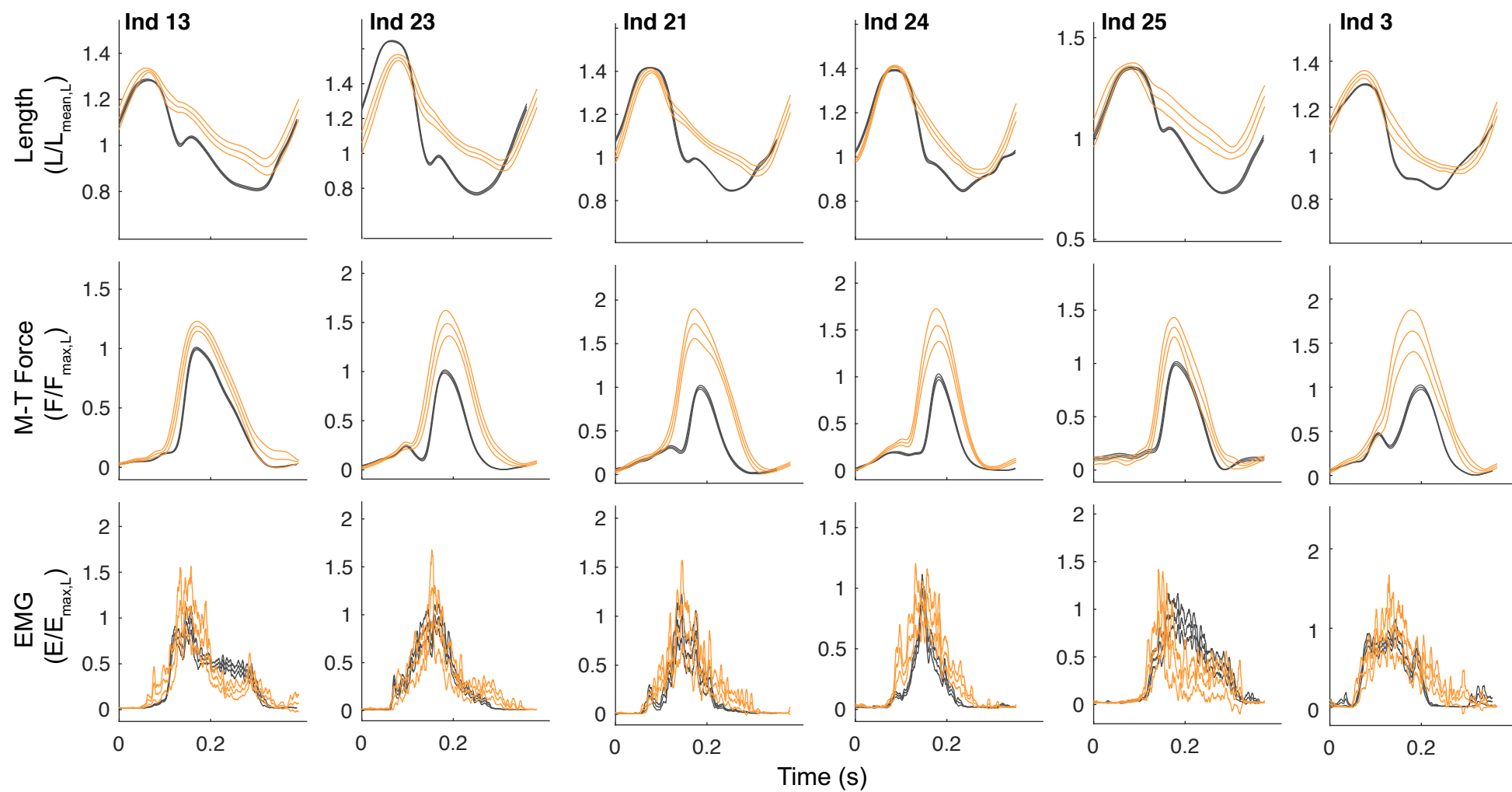


Figure 8

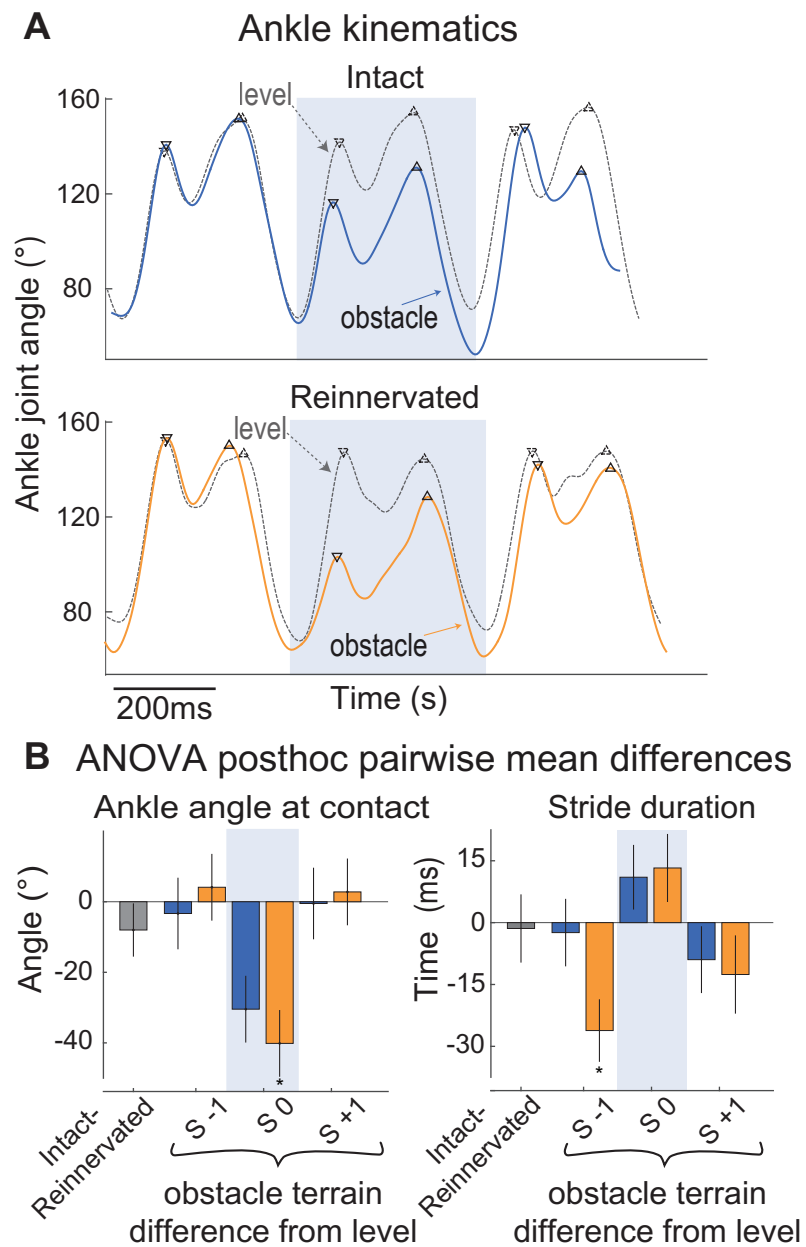


Figure 9

