Supplemental Information

Neural Interfacing Architecture Enables Enhanced Motor Control and Residual Limb Functionality Postamputation

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Supplemental Video 1: AMI construct activation
Supplemental Video 2: Fascicle Dynamics during phantom ankle movement in TB and AB subjects
Supplemental Video 3: Fascicle Dynamics during phantom ankle movement in the AMI
Supplemental Video 4: Ankle AMI movement visualized at the synovial canal during cycles of plantarflexion-dorsiflexion
Supplemental Video 5: Subtalar AMI movement visualized at the synovial canal during cycles of inversion-eversion.
Fig S1. A) Individual muscles of a residuum incorporating AMIs were palpated and marked using a skin marker to guide electrode placement. The locations for electrodes corresponding to the LG and TP are visualized on the anterior medial view. B) Anterior view of the limb shows representative localization of the ankle and subtalar AMIs with respect to bony landmarks. C) Anterior lateral view shows the locations for electrodes corresponding to the TA and PL muscles. D) Anterior view of the residuum shows a grid of 64 electrodes positioned over the muscles. E) Lateral view of residuum showing recording electrodes.
Fig S2. A) A two-axis goniometer was secured to the posterior aspect of the unaffected ankle to capture the angles of the contralateral ankle and subtalar joints, which mirrored the movements of the phantom during all trials. B) Subjects were positioned in a supine position with their lower leg elevated on a trapezoidal pillow to minimize motion of the electrodes and wiring against the leg, while still allowing for free and full movement of the unaffected foot. C) Subjects performed positional discrimination tasks by following the commands displayed on the screen, with a pre-recorded soundtrack. Tasks required activating the appropriate muscles in the residual limb to move the phantom limb to directed positions without direct visualization of either limb. The subjects mirrored each movement on their intact limb.
Fig. S3. A) Ultrasound probe was placed on the TA to capture coupling of the TA-LG and measure fascicle strain in the TA. B) Ultrasound probe was placed transversely over the tibial flat to capture the movement of each AMI construct through its synovial tunnel. C) Processing of ultrasound data through UltraTrack enabled the measurement and tracking of muscle fascicle length. D) Representative sample of fascicle length tracking in the TA during cyclic PF of the ankle AMI. E,F) Imaging of the TA located the tantalum beads, which moved with contraction. The beads’ translated positions are visualized in F. G) The transverse view of the synovial canal exhibited the beads in the synovial tunnel as well as in the TA muscle.
**Fig S4. Pain Scores** Left) Boxplot of the phantom pain scores from the AB cohort demonstrated that the post-operative scores were significantly lower than the preoperative scores (*p < 0.005, student’s t-test, varying sample sizes). Right) Table of the individual subject scores. Many subjects were non-responsive to the survey at given timepoints or had not reached the time point. Two scores were outliers for the following reasons: *Subject 1 experienced severe pain due to a broken tibia that was later revised. **Subject 3 experienced extreme pain with the intact foot at the time of the survey.
Fig. S5. A) Relationship between fascicle strain and ROM for AB subjects (green) and TB subjects (blue) during plantarflexion is described by a logarithmic function. B) Relationship between fascicle strain and ROM for AB subjects (green) and TB subjects (blue) during dorsiflexion is described by a logarithmic function. C) Relationship between fascicle strain and phantom sensation for AB subjects (green) during plantarflexion is described by a linear relationship. No significant correlation between parameters exists in TB subjects (blue). D) Relationship between fascicle strain and phantom sensation for AB subjects (green) during dorsiflexion is described by a linear relationship. No significant correlation between parameters exists in TB subjects (blue).
Fig. S6. P values for the F-test from the positional differentiation data are plotted for each subject in the AB (green, n = 14) and TB (blue, n=6) cohorts. The asterisk denotes a significant difference (p < 0.0125) between groups. Independence between commanded activation levels was compared between cohorts using multivariate analysis of variance (ANOVA), Bonferroni corrected for multiple comparisons at a statistical significance alpha of p < 0.008. Subjects in the AB group demonstrated significantly less variance in performing the task for each cardinal direction (PF, DF, IN, EV). This is consistent with data shown for the median performer shown in Fig. 3A and Fig. 3B.