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Real-time biofeedback integrated into neuromuscular training reduces high-risk knee biomechanics and increases functional brain connectivity: A preliminary longitudinal investigation

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Abstract

Prospective evidence indicates that functional biomechanics and brain connectivity may predispose an athlete to an anterior cruciate ligament injury, revealing novel neural linkages for targeted neuromuscular training interventions. The purpose of this study was to determine the efficacy of a real-time biofeedback system for altering knee biomechanics and brain functional connectivity. Seventeen healthy, young, physically-active female athletes completed six weeks of augmented neuromuscular training (aNMT) utilizing real-time, interactive visual biofeedback and 13 served as untrained controls. A drop vertical jump and resting state functional magnetic resonance imaging were separately completed at pre and post time points to assess sensorimotor adaptation. The aNMT group had a significant reduction in peak knee abduction moment (pKAM) compared to controls ($p = .03$, $d = .71$). The aNMT group also exhibited a significant increase in functional connectivity between the right supplementary motor area and the left thalamus ($p = .0473$ after false discovery rate correction). Greater percent change in pKAM was also related to increased connectivity between the right cerebellum and right thalamus for the aNMT group ($p = .0292$ after false discovery rate correction, $r^2 = .62$). No significant changes were observed for the controls (p 's $> .05$). Our data provide preliminary evidence of potential

neural mechanisms for aNMT-induced motor adaptations that reduce injury risk. Future research is warranted to understand the role of neuromuscular training alone and how each component of aNMT influences biomechanics and functional connectivity.

1. Introduction

Risk of musculoskeletal injury, specifically anterior cruciate ligament (ACL) injury, is associated with decreased neuromuscular control and coordination during dynamic activities (Griffin et al., 2000; Hewett et al., 2005; Hewett, Torg, & Boden, 2009; Ireland, 2002; Krosshaug, Slauterbeck, Engebretsen, & Bahr, 2007; Zazulak, Hewett, Reeves, Goldberg, & Cholewicki, 2007). Advances in 3D motion analyses and force platforms have permitted the assessment of neuromuscular coordination using the drop vertical jump (DVJ) (Chaudhari et al., 2007; Cruz et al., 2013; DiCesare, Kiefer, Bonnette, & Myer, 2019; Doherty et al., 2016; Earl, Monteiro, & Snyder, 2007; Etnoyer, Cortes, Ringleb, Van Lunen, & Onate, 2013; Ford, Myer, & Hewett, 2003, 2007a; Hewett et al., 2005; Limroongreungrat & Boonkerd, 2019; McLean et al., 2007; Paterno et al., 2010; Popovic et al., 2018; Schmitz et al., 2014; Taylor et al., 2017). During the landing phase of the DVJ, tri-planar hip, knee, and ankle motion that results in the knee collapsing toward the midline, or 'dynamic valgus,' is quantified via an external peak knee abduction moment (pKAM) (Carson & Ford, 2011; Ford, Myer, & Hewett, 2007b; Ford et al., 2005; Ford, Shapiro, Myer, Van Den Bogert, & Hewett, 2010; Galloway et al., 2018; Hewett et al., 2005; Malfait et al., 2014; Myer et al., 2010; Myer et al., 2013; Myer et al., 2015). Prospective biomechanical measures of pKAM during landing predict non-contact ACL injury with 73% specificity and 78% sensitivity (Hewett et al., 2005), a finding that has motivated ACL injury prevention programs to improve neuromuscular coordination through neuromuscular training (NMT) (Ireland, 2002; Myer, Chu, Brent, & Hewett, 2008; Sugimoto, Myer, Foss, & Hewett, 2014, 2015; Sugimoto, Myer, McKeon, & Hewett, 2012). However, traditional ACL injury prevention programs are susceptible to non-compliance (Sugimoto, Myer, Bush, et al., 2012), require considerable resources and trained instructors (Hewett, Ford, & Myer, 2006), and have poor dose-response relationships (Sugimoto, Myer, McKeon, et al., 2012) thus requiring high compliance and extended training durations to elicit desirable movement outcomes (Padua et al., 2012). These barriers may explain why ACL injury incidence rates remain high (Beynnon

et al., 2014; Sanders et al., 2016) and highlight a need for complementary approaches that enhance athlete compliance and widespread implementation. As motivation appears to be a critical factor for achieving high compliance (Steffen, Myklebust, Olsen, Holme, & Bahr, 2008), a stronger mechanistic understanding of NMT may improve ‘buy-in’ (i.e., increase motivation) to facilitate implicit processes underlying enhanced motor learning (Wulf & Lewthwaite, 2016).

One mechanism that has been historically overlooked regarding NMT is how the central nervous system (CNS), specifically the brain, contributes to ACL injury (Grooms & Onate, 2016). ACL injuries often occur during scenarios that require intricate sensorimotor coordination (Krosshaug, Nakamae, et al., 2007), which exemplify the critical role of the CNS in maintaining functional joint stability. In an exploratory neuroimaging study (Diekfuss, Grooms, Yuan, et al., 2019), females who sustained complete ACL ruptures during their competitive soccer season were matched to healthy teammates. Prospective functional magnetic resonance imaging (fMRI) revealed reduced correlation in blood oxygen level dependent (BOLD) signal activity at rest (i.e., functional connectivity) between the left primary somatosensory cortex (a region that processes sensory nervous system information) and the right posterior lobe of the cerebellum (a region associated with motor control) prior to the ACL injury (Diekfuss, Grooms, Yuan, et al., 2019). Hemispheric lateralization of significant findings (i.e., right versus left cerebellum) could be due to the small sample size in the prior study, precluding the power to detect additional effects, or possibly due to lower limb dominance, which was not reported (Diekfuss, Grooms, Yuan, et al., 2019). Nevertheless, somatosensory-cerebellar connectivity throughout both hemispheres is vital for successful motor coordination and navigation (Manto et al., 2012) and these data identified a potential neural predisposition to ACL injury that could be investigated, and possibly modified, through further research (Diekfuss, Grooms, Yuan, et al., 2019). While ACL injury prevention training has been shown to reduce *corticomotor excitability* in the gluteus maximus relative to strength training (using transcranial magnetic stimulation) (Powers & Fisher, 2010), the effects of traditional ACL injury prevention training on *brain functional connectivity* has not been clearly established. Previous research has demonstrated that other forms of motor skill training, ranging from discrete fine motor tasks such as finger tapping (Ma, Narayana, Robin, Fox, & Xiong, 2011) and tool use (i.e., chopstick handling) (Yoo, Sohn, & Jeong, 2013) to more complex balance board training (Taubert, Lohmann, Margulies, Villringer, & Ragert, 2011), drum training (Amad et al., 2016), and aerobic fitness training (Voss et al., 2010), have

pronounced effects on functional connectivity. Considering its behavioral relevance and the sensitivity of measures of functional connectivity to changes within the CNS (Guerra-Carrillo, Mackey, & Bunge, 2014), functional connectivity could be a valuable tool to supplement our understanding of ACL injury prevention.

In one preliminary study investigating the role of ACL injury prevention training in relation to high-risk knee biomechanics and neuroplasticity (Grooms et al., 2018), healthy athletes completed six weeks of standard NMT supplemented with real-time biofeedback, or augmented NMT (aNMT). Participants completed pre- and post-training fMRI sessions employing a knee motor control testing paradigm and were separately assessed in 3D motion capture using a virtual-reality simulated soccer scenario for high-risk knee biomechanics (Grooms et al., 2018). aNMT increased sensory and motor planning network activity and decreased motor cortex activity (suggesting improved efficiency), and both neural changes were correlated with a reduction in high-risk knee biomechanics (Grooms et al., 2018). These preliminary data demonstrated neuroplasticity potentially resulting from aNMT and highlighted the need to further examine the adaptability of brain connectivity that may underlie ACL injury risk (Diekfuss, Grooms, Nissen, et al., 2019; Diekfuss, Grooms, Yuan, et al., 2019).

The purpose of the present study was to assess the efficacy of aNMT for improving high-risk landing biomechanics and for increasing sensorimotor, knee-related brain functional connectivity. We hypothesized that (1) aNMT would improve knee biomechanics that are high risk during the DVJ, (2) participation in aNMT would increase sensorimotor functional connectivity between regions related to knee motor control, and (3) improvements in knee biomechanics that are high risk would be associated with increased knee-related, sensorimotor functional connectivity for those who participated in aNMT.

2. Method

As changes in biomechanics and functional connectivity as a function of aNMT have yet to be explored, we used prior, related research to guide our sample size. Specifically, one study examined changes in functional connectivity following 13 sessions (once session per week for 13 weeks) of combined coordinative, cognitive, and visual training (Demirakca, Cardinale, Dehn, Ruf, & Ende, 2016). The authors found significant pre- to post-training functional connectivity changes using an n of 32 ($n = 21$ training & $n = 11$ controls) (Demirakca et al., 2016). Another

study (Amad et al., 2016) found significant functional connectivity changes following 24 sessions of drum training (three sessions per week for eight weeks) with an n of 31 ($n = 15$ training, $n = 16$ controls). As drumming is a cardiovascular-demanding bilateral coordinative exercise (De La Rue, Draper, Potter, & Smith, 2013) (comparable to NMT more generally) and the study by Demirakca et al. (2016) was multimodal (i.e., cognitive, visual, and coordinative demands akin to aNMT), we aimed to enroll a similar number of participants in this preliminary investigation ($n \sim 30$).

Before engaging in a larger scale, resource-intensive and costly randomized controlled trial, we completed this proof-of-concept preliminary investigation (non-randomized controlled trial) to determine potential efficacy for aNMT to improve biomechanical and functional connectivity. One local high-school volunteered to participate in our proposed study and enrolled 25 female soccer players. Eight of these athletes were not included in the present analyses due to MRI contraindications/poor imaging data (e.g., metal orthodontics/artifact/technical issue). The participants who did not receive (or have usable) neuroimaging data will be presented in a companion manuscript focused on biomechanics only. Raw demographic data for the final 17 participants used in all analyses herein (aNMT group) is presented in **Table 1**.

To enhance the study's research design, we contacted a second high-school (located in the same city as the first high-school) to participate in two neuroimaging sessions separated by approximately seven weeks (i.e., to serve as controls). Thirteen female soccer athletes from the second high-school volunteered to participate and enrolled as controls. These control participants did not differ in age, height, or weight to that of the aNMT group (all $p > .05$), and their raw demographic data is also presented in **Table 1**.

“INSERT TABLE 1 ABOUT HERE”

The study took place during both high-schools' soccer offseason, data from both groups were collected simultaneously, and all participants received equal compensation. No participants from either group were involved in any structured conditioning or other ACL prevention programs over the duration of the study period. All participants ($n = 30$) completed DVJ testing using 3D motion capture and a resting-state fMRI (rs-fMRI) scan at a pre- and post-test time point separated by approximately seven weeks (the aNMT group underwent a six week

intervention described below). The institutional ethics committee approved the project and informed consent or assent (if under 18) was obtained from all participants prior to commencing the study.

2.1 Augmented Neuromuscular Training (aNMT)

The aNMT biofeedback group completed six weeks of standard NMT (3 times per week; 18 total sessions) as part of a pre-season conditioning program (Myer, Ford, Palumbo, & Hewett, 2005). NMT consisted of plyometric, core strengthening and balance, resistance, and speed training that are detailed in Myer et al. 2005. NMT sessions were team-based (group training), were provided at no cost to the athlete, and lasted approximately 1.5 hours (45 minutes per NMT station \times two stations per session [e.g., plyometric and speed training]). Mean compliance for NMT was 85.0% (+/- 11.8%). The majority of these NMT sessions were further supplemented with an additional exercise that involved participants interacting with a real-time visual biofeedback stimulus (i.e., aNMT) performed on average two times per week for six weeks over the course of the program (i.e., 12 of the total 18 sessions). The aNMT biofeedback protocol consisted of six movements (squat, pistol squat, single-leg romanian dead lift, overhead squat, squat jump, and tuck jump). Participants' completed each exercise twice (2 separate aNMT sessions) while interacting with the aNMT stimulus for three sets of 10 repetitions for bilateral exercises (squat, overhead squat, squat jump, tuck jump) and three sets of five repetitions per leg for the unilateral exercises (pistol squat, single-leg romanian dead lift). aNMT was run individually for each subject (also at no cost to the athlete) and required an additional 15 minutes to complete. If a participant was absent from the aNMT session (i.e., missed a NMT session that included aNMT), the participant would complete the aNMT session during a subsequent NMT session, such that compliance for aNMT was 100%.

The following text provides further information regarding the aNMT stimulus, but we direct the reader to recent literature for in-depth methodological details (Bonnette et al., 2020; Bonnette, DiCesare, Kiefer, et al., 2019). Briefly, the aNMT stimulus consisted of a dynamic, rectangular shape with vertices that were mapped to key biomechanical variables related to ACL injury risk (Ford, Myer, Schmitt, van den Bogert, & Hewett, 2008; Hewett et al., 2005; Hewett et al., 2009; Myer et al., 2009; Paterno, 2017; Schoenfeld, 2010; Zazulak et al., 2007). These variables included lateral trunk flexion, knee to hip joint extensor moment force ratio, knee abduction moment of force, vertical ground reaction force ratio, and foot center of pressure

location. The stimulus shape responded in real time as a function of participants' movements as their actions led to changes in those biomechanical variables. Variables that were possible to calculate during all six aNMT exercises, such as lateral trunk flexion, were included in each exercise's aNMT stimulus. When it was not possible to calculate a variable during some aNMT exercises, such as the vertical ground reaction force ratio during the pistol squat and single leg romanian dead lift (unilateral exercises), the variable was not included as part of the aNMT stimulus. Participants were instructed to perform all exercises to achieve a goal rectangle shape, which equated to producing biomechanical parameters associated with ACL injury risk reduction. Deviations toward injury risk factors resulted in specific shape distortions (Figure 1). The variables used for each aNMT session were identical for all participants and did not vary based on an individual participant's performance. Note that the aNMT stimulus presented in Bonnette, DiCesare, Kiefer, et al. (2019) and Bonnette et al. (2020) was wirelessly transmitted in real-time to video eyeglasses worn by participants, whereas the aNMT stimulus used in the present study was displayed on a projector screen (Grooms et al., 2018).

“INSERT FIGURE 1 ABOUT HERE”

Though the equipment required to produce the aNMT stimulus are not readily accessible outside of a research laboratory at this time (e.g., force plates, 3D motion capture system), ongoing research aims to implement aNMT with new, cost-effective technologies while maintaining similar efficacy and validity of biofeedback. For instance, portable, wireless, and markerless motion analysis systems are commercially available for less than a few thousand dollars (e.g., Microsoft Azure Kinect; Microsoft Corp., Redmond, WA), and efforts are being made to bring 3D motion capture capabilities to consumer devices such as the iPad and iPhone (Apple Inc, Cupertino, CA). Likewise, kinetic data can be obtained from portable force plates (e.g., BODITRAK, BodiTrak Sports, Webster Groves, MO) at lower costs than traditional lab setups. Integration of data across systems could support a real-time biofeedback aNMT stimulus to be presented using standard visual displays (e.g., computer monitors). With respect to NMT more generally, previous literature has reported estimated costs of ACL prevention programs to range from a few dollars to ~ \$500.00 (after adjusting for inflation; consumer price index) per athlete per season (Hewett et al., 2006), depending on program specifics (e.g., number of

sessions, personnel for training). Incorporating emergent cost-effective technologies with NMT to display real-time biofeedback will also allow for personalized training at a further reduced cost (e.g., by removing the need for expensive clinicians to provide feedback) for easier implementation in non-laboratory settings (e.g., at home, high school gymnasium). However the authors acknowledge that continued research to test these technologies against gold standard 3D motion analysis systems and longitudinal efficacy clinical trials are needed.

2.2 Drop Vertical Jump

To assess whether aNMT improved high-risk knee biomechanics based on the DVJ (Hypothesis 1), participants were outfitted with 31 retroreflective markers and completed a standing trial with all joints in neutral position (Grooms et al., 2018). Participants were then asked to stand on top of a box (31 cm high) with their feet positioned 35 cm apart and instructed to drop directly down off the box and immediately perform a maximum vertical jump, raising both arms while jumping for an overhead target, for three trials (Ford et al., 2003). Marker trajectory and ground reaction force data were recorded using a 44-camera, high-speed (240 Hz) digital motion analysis system (Motion Analysis Corp.) and two embedded force platforms (AMTI, Watertown, MA), post-processed with Visual3D (C-Motion, Inc., Germantown, MD), and analyzed using custom MATLAB scripts (MathWorks, Inc, Natick, MA). Greater pKAM during landing is associated with increased ACL injury risk (Hewett et al., 2005) and therefore pKAM was used as the biomechanical dependent variable of interest. We summed the three pKAM values for each limb (six total) and computed a single, bilateral average (by dividing the summed value by six). Our rationale for using a bilateral average (rather than separate unilateral analyses) was that our complementary dependent variable of interest (functional connectivity) was collected during the resting state (no unilateral active movement to drive a lateralized brain response) and both limbs were equivocally trained during aNMT (primarily bilateral exercises; unilateral exercises were repeated for both limbs).

2.3 Neuroimaging

MRI was conducted on a Phillips 3T Ingenia scanner (Philips Medical Systems, Best, the Netherlands) with a 32-channel, phased array head coil. A magnetization-prepared rapid gradient-echo sequence was used to acquire a high resolution 3D T1-weighted images (sagittal): TR = 8.1 ms, TE = 3.7 ms; field of view = 256 × 256 mm; matrix= 256 × 256; in-plane resolution = 1 × 1 mm; slice thickness = 1 mm; number of slices = 180. To test whether aNMT

influenced brain sensorimotor functional connectivity related to knee motor control based on resting-state fMRI (Hypothesis 2), participants were asked to look at a crosshair displayed on a projector screen and remain still for approximately 5.5 minutes. Resting-state fMRI data were acquired with the following parameters: TR = 650 ms; TE = 30 ms; flip angle = 53°; field of view = 200 × 200 mm; acquisition matrix = 68 × 66; SENSE factor = 1.5; reconstructed in-plane resolution = 2.5 × 2.5; slice thickness = 3.5 mm; number of slices = 40; multi band factor = 4.

Processing of the rs-fMRI data were performed using the CONN toolbox (version 17.F, <http://www.nitrc.org/projects/conn>) (Whitfield-Gabrieli & Nieto-Castanon, 2012). The CONN toolbox utilizes the Statistical Parametric Mapping (SPM) 12 package (Wellcome Institute of Cognitive Neurology, London) for spatial preprocessing which includes realignment and unwarping, normalization to Montreal Neurological Institute (MNI) template space (resampled to 2 mm isotropic), and smoothing (8 mm full-width half-max kernel). Temporal preprocessing steps were completed in CONN and included scrubbing of motion-outlier frames (> 0.9 mm or > ±5 standard deviation in global signal) and regression of the top five principle components of the BOLD signal from cerebrospinal fluid and white matter compartments (aCompCor) (Behzadi, Restom, Liao, & Liu, 2007), as well as zero- and first-order derivatives of realignment parameters and band-pass filtering to a window of 0.008 Hz - 0.09 Hz.

To examine whether aNMT influenced sensorimotor functional connectivity specifically related to knee motor control (Hypothesis 2) and whether such functional connectivity changes were related to improvements in high-risk knee biomechanics (Hypothesis 3), 25 regions of interest (ROI) (10 mm spheres) were derived from previous studies that utilized a knee flexion/extension paradigm (Grooms, Page, & Onate, 2015; Kapreli et al., 2007). Development of these ROIs (left and right sensorimotor-related regions) has been previously described in detail (Diekfuss, Grooms, Yuan, et al., 2019) and were selected due to their prospective relation to ACL injury (Diekfuss, Grooms, Nissen, et al., 2019; Diekfuss, Grooms, Yuan, et al., 2019). Fisher-transformed Pearson correlation coefficients between the average residual BOLD signal time series (extracted from the individual ROIs) were used to define functional connectivity (Biswal, Zerrin Yetkin, Haughton, & Hyde, 1995).

2.4 Data analyses

First, an independent *t*-test was conducted to assess any significant differences in pKAM at the pre time point. To address Hypothesis 1, percent change scores for the pKAM (pre to post) were then calculated for each group. One outlier was observed for the aNMT group (box plot inspection revealed this subject's data point was outside 1.5 times the interquartile range [i.e., below the lower whisker]). As this was the only outlier (and this participant had usable neuroimaging data), we replaced this value with the group mean (Tabachnick & Fidell, 2007). Percent change scores for each group were then compared using an independent *t*-test.

To test hypothesis 2, we identified appropriate ROIs to use as 'seed' ROIs from the total 25 used previously (Diekfuss, Grooms, Nissen, et al., 2019; Diekfuss, Grooms, Yuan, et al., 2019). Twenty of the ROIs were initially derived from knee flexion/extension movements within a population that excluded those with a history of musculoskeletal injury (Kapreli et al., 2007), whereas five ROIs were derived from the same knee/flexion paradigm but in a subject who previously had an ACL injury and went on to a second ACL injury (Grooms et al., 2015). As the present study did not track actual ACL injury incidence or study those with a history of ACL injury, we elected to use the 20 ROIs from Kapreli et al. (2007) as seeds, but still included the five ROIs from Grooms et al. (2015) as target ROIs in the analysis. The anatomical ROIs were identified and an omnibus *F*-test was conducted to determine which of the 20 seed ROIs exhibited significant connectivity differences with one or more of the other 24 ROIs (inclusive of all seed and target ROIs) for the aNMT and control groups, independently. Specifically, one-way ANOVAs were used to identify 'seed' ROIs that exhibited a significant group effect in their connectivity patterns. Post-hoc paired-samples *t*-tests were applied to each pairwise connection between any significant seed ROIs and each other ROI to determine specific pre to post connectivity changes for each group, independently. Alpha level was set at $p < .05$ and multiple comparison error corrections using the false discovery rate (FDR) approach were used for all *F*- and *t*- tests (Chumbley, Worsley, Flandin, & Friston, 2010).

For hypothesis 3, linear regression analyses were conducted to determine whether percent change in pKAM was related to changes in sensorimotor connectivity related to knee motor control. Percent change in pKAM was used as the predictor variable for the pre to post change in functional connectivity for all 25 possible ROI-to-ROI comparisons (300 regressions total) for each group, independently. We included all possible ROI-to-ROI comparisons (including the five ROIs from Grooms et al. 2015) as pKAM is more directly related to ACL injury risk. The

FDR approach was used to correct for multiple comparisons set at $p < .05$ (Chumbley et al., 2010). In other words, this analysis assessed whether the percent change in pKAM (the predictor variable) was associated with pre to post changes in functional connectivity (the predicted variable) for any ROI-to-ROI comparison, with only the relationships surviving the FDR correction being deemed as significant/meaningful. Based on prior literature, we expected our aNMT group to improve biomechanics (Bonnette et al., 2020; Bonnette, DiCesare, Kiefer, et al., 2019; Ericksen et al., 2016; Ericksen, Thomas, Gribble, Doebel, & Pietrosimone, 2015; Ford, DiCesare, Myer, & Hewett, 2015) and, as the strength of resting-state functional connectivity reflects a history of co-activation during active states (Corbetta, 2012; Di, Gohel, Kim, & Biswal, 2013), we expected sensorimotor, knee-related connectivity to strengthen following aNMT, since the aNMT exercises engaged the knee akin to the task-based knee fMRI studies used to derive the ROIs (Grooms et al., 2015; Kapreli et al., 2007). Thus, all statistical analyses noted above were conducted using one-tailed tests. For any significant finding we also included the uncorrected p value when relevant (i.e., the raw p value [$p^{\text{uncorrected}}$] before the application of the conservative FDR p value correction used for the connectivity analyses [$p^{\text{corrected}}$]), means and SDs, and effect sizes to facilitate reader interpretation regarding significance.

3. Results

As noted in **Table 1**, no significant differences in pKAM at the pre time point were observed between the two groups ($p > .05$). As shown in Figure 2, the percent change in pKAM was significantly greater (i.e., peak knee moment decreased) for the aNMT group ($M = 31.80$, $SD = 36.53$) compared to the control group ($M = 2.57$, $SD = 46.78$), $t(28) = 1.92$, $p = .03$, $d = .71$. The omnibus test revealed significant connectivity changes from pre- to post for the left thalamus and all other target ROIs for the aNMT group, $F(4, 13) = 7.52$, $p^{\text{uncorrected}} = .0023$, $p^{\text{corrected}} = .0460$. Post-hoc analyses revealed significantly increased connectivity between the left thalamus and the right supplementary motor area (SMA) for the aNMT group from pre ($M = -.10$, $SD = .17$) to post ($M = .07$, $SD = .18$), $\beta = .17$, $t(16) = 3.37$, $p^{\text{uncorrected}} = .0020$, $p^{\text{corrected}} = .0473$. Our regression analyses revealed that greater improvement in the percent change in pKAM was related to increased connectivity at post between the right cerebellum (anterior division) and right thalamus for the aNMT group, $t(15) = 4.89$, $p^{\text{uncorrected}} = .0001$, $p^{\text{corrected}} = .0292$, $r^2 = .61$ (see Figure 3). No significant pre to post changes in functional connectivity or associations between

percent change in pKAM and pre to post change in functional connectivity were observed for the controls (all $p^{\text{corrected}} > .05$).

“INSERT FIGURE 2 ABOUT HERE”

“INSERT FIGURE 3 ABOUT HERE”

4. Discussion

Our biomechanical results are consistent with previous reports demonstrating the capability for altering knee biomechanics while interacting with an aNMT stimulus (Bonnette et al., 2020; Bonnette, DiCesare, Kiefer, et al., 2019) and following six weeks of aNMT (Grooms et al., 2018). Considering depressed functional connectivity within the knee motor network has been noted as a potential neural target to supplement ACL injury prevention (Diekfuss, Grooms, Nissen, et al., 2019; Diekfuss, Grooms, Yuan, et al., 2019), it is noteworthy that our aNMT group, who interacted with a visual stimulus during 6 weeks of training, did significantly increase cortical (SMA)-thalamic connectivity and experienced greater improvements in pKAM that were associated with increased cerebellar-thalamic connectivity. The SMA has been extensively linked to motor control as it is important for initiating and generating movement (Tanji & Hoshi, 2001). The thalamus—traditionally viewed as a ‘brain relay hub’—maintains a critical role in motor control due to its anatomical location between cortical and subcortical motor-related areas, (Bosch-Bouju, Hyland, & Parr-Brownlie, 2013) and the cerebellum is also vitally important to motor control as it contributes to timing of motor activity (Manto et al., 2012).

Resting-state functional connectivity reflects a history of co-activation of spatially distinct brain regions during active states (Corbetta, 2012; Di et al., 2013), which provides a framework for our aNMT-related connectivity findings. The thalamus receives and transmits sensory information from many cortical regions, including the SMA (Rouiller, Tanne, Moret, & Boussaoud, 1999), which plausibly increased activity in response to the sensorimotor demands of aNMT. Neurophysiological studies have demonstrated that integrating two sensory modalities (in our case proprioception and vision) within the same spatially relevant context (i.e.,

crossmodal integration) involves distinct neural processes (Calvert, 2001). Training with the aNMT biofeedback stimulus required a high degree of cross-modal integration, as the visual feedback changed in real-time with proprioceptive information, which may have strengthened cortical-thalamic connectivity. Neural networks involved in crossmodal operations often include the superior temporal sulcus, the intraparietal sulcus, frontal cortex, parietal regions, and insular/ claustrum regions (Calvert, 2001; Calvert, Hansen, Iversen, & Brammer, 2001; Di et al., 2013; Lewis, Beauchamp, & DeYoe, 2000; Macaluso & Driver, 2001). Although our *a priori* knee network ROI approach precluded investigation into all of the aforementioned regions, our findings of strengthened cortical-thalamic connectivity post-aNMT provide preliminary support that crossmodal integration may be the driving mechanism for aNMT. More specifically, we hypothesize that aNMT may facilitate improved motor planning within the SMA through crossmodal, sensory relay processing enhancements mediated by the thalamus. An interesting area of future investigation may be to evaluate how altered movement mechanics measured during the presentation of the aNMT stimulus (e.g., using a heat map analysis (Bonnette et al., 2020; Bonnette, DiCesare, Kiefer, et al., 2019) contribute to crossmodal-related brain activity in real-time (e.g., using portable electroencephalography).

Our pre to post regression analyses revealed that changes in knee biomechanics were related to strengthened *cerebellar*-thalamic connectivity for the aNMT group, rather than *cortical*-thalamic connectivity. On the surface these results may appear divergent, but are likely explained by task constraints. The DVJ is a dynamic, ballistic task that requires a high degree of precision, motor timing, and motor coordination on the part of the athlete that was tested separately from aNMT. The cerebellum contributes to feedforward mechanisms (Pisotta & Molinari, 2014) and sensorimotor timing (Spencer & Ivry, 2013), and may be more involved in the demands of the DVJ, than in than the slower, aNMT-specific NMT exercises. In other words, changes in pKAM as measured by the DVJ may be more sensitive to changes in cerebellar-thalamic connectivity, and may be less related to the above-mentioned crossmodal neural operations occurring during aNMT.

Biofeedback systems are driven by their capability to induce implicit motor learning strategies (i.e., absent of specific explicit instruction) (Bonnette, DiCesare, Diekfuss, et al., 2019; Bonnette et al., 2020; Bonnette, DiCesare, Kiefer, et al., 2019). Implicit learning contributes to reorganization of the motor cortex, (Hirano, Kubota, Koizume, Tanaka, & Funase, 2017)

highlighting potential strategies to leverage neuroplasticity. For instance, supplementing ACL injury prevention methods with an external focus (i.e., verbal instruction that directs attention towards the effects of one's movement (Diekfuss, Rhea, et al., 2019; Wulf, 2013; Wulf, Höß, & Prinz, 1998)) and/or integrating analogies (i.e., verbal instruction utilizing metaphors (Liao & Masters, 2001)) may enhance functional connectivity through synaptogenic processes (Wulf & Lewthwaite, 2016). Indeed, recent evidence indicates that adopting such motor learning techniques does in fact influence brain activity (Raisbeck, Diekfuss, Grooms, & Schmitz, 2019; Sakurada, Hirai, & Watanabe, 2019). Capitalizing on such implicit processes to enhance motor learning may be especially beneficial for ACL injury prevention methods to engage cortical-thalamic and/or cerebellar-thalamic neural processes. Many musculoskeletal prevention and rehabilitation efforts focus on *motor* progression using strength training, proprioceptive exercises, range of motion activities, etc. (Arderm et al., 2018; Cavanaugh & Powers, 2017). Motor progression success is often assessed through biomechanical assessment (Hopper, Haff, Joyce, Lloyd, & Haff, 2017; Myer et al., 2005), and while current NMT practices are often beneficial in reducing risk of ACL injury (Petushek, Sugimoto, Stoolmiller, Smith, & Myer, 2019), our data support that *neural* progression should also be considered as part of current standards of care (Gokeler, Neuhaus, Benjaminse, Grooms, & Baumeister, 2019). Clinicians could potentially leverage motor learning principles (Gokeler et al., 2019; Lewthwaite & Wulf, 2017; Wulf & Lewthwaite, 2016) to engage the appropriate neural networks distinct to various musculoskeletal ailments (Diekfuss, Grooms, Nissen, et al., 2019; Diekfuss, Grooms, Yuan, et al., 2019; Grooms et al., 2017; Silfies, Vendemia, Beattie, Stewart, & Jordon, 2017).

The implicit motor learning strategies that we hypothesize underlie aNMT may also shed light on laterality within the functional connectivity findings. The increase in cortical-thalamic connectivity was localized to the right SMA and left thalamus, whereas the increase in cerebellar-thalamic connectivity was localized to the right hemisphere, only. As the thalamus is important for transmitting information throughout the entire cortex (Sherman & Guillery, 2002), it is interesting that strengthened left *and* right thalamic connectivity was localized to right sensorimotor regions. Though a clear behavioral role for each hemisphere is inconclusive (i.e., right versus left brain dominance) (Corballis, 2014), there is accumulating evidence that the right hemisphere is particularly involved in intuition and 'insight-based' functions (McCrea, 2010), which are highly related to implicit learning in general (Lieberman, 2000). Though cognitive-

based measures of intuition were not obtained, many nonverbal coding skills (e.g., processing the tone of a voice) have been shown to rely heavily on networks and associated neural substrates primarily within the right hemisphere (Borod, 1993; Lieberman, 2000; McCrea, 2010). aNMT abstractly displays information that an athlete must use to learn how to coordinate multiple biomechanics variables without explicit instruction (Bonnette et al., 2020; Bonnette, DiCesare, Kiefer, et al., 2019). The aNMT group may have steadily improved their ability to intuitively ‘code’ the perceptual information displayed by the aNMT stimulus, resulting in hyperactive right hemispheric activity that co-activated with the thalamus throughout the intervention. Though lesion and fMRI studies provide neurological data in favor of the right hemisphere for such intuition-based functionality (Goel & Vartanian, 2004; Miller & Tippett, 1996), we stress that such an interpretation is largely speculative. A reasonable alternative explanation could be that participants in the aNMT group varied in terms of right and left side motor dominance (right versus left handedness and footedness) that drove some of the observed effects. Unfortunately, handedness and footedness were not collected in this study, and we emphasize that future large-scale trials should collect these data to further deconstruct the neural mechanisms underlying aNMT-driven biomechanical changes.

4.1 Limitations

While our study provides the first report combining biomechanical and functional connectivity changes that occur following aNMT, the present study is not without its limitations (including our noted failure to include measures of athlete handedness and footedness). First, our control group did not complete a standard NMT (without real-time biofeedback) to deconstruct whether our observed biomechanical and connectivity changes were due to the biofeedback stimulus or NMT, more generally. Second, we elected to examine pre to post changes in connectivity and the associated relationships with biomechanical adaptations independently for each group (rather than longitudinal group-wise differences using ANOVAs) and acknowledge this may not be the strongest statistical model as no interaction effects were examined. However, this approach was chosen to identify proof-of-concept, *preliminary* neural mechanisms (due to a low sample size) that could guide future, large-scale randomized controlled clinical trials that optimize aNMT for enhanced brain adaptation. Lastly, participants were not randomized to the aNMT or control group at the participant level (one school enrolled in aNMT and a second school enrolled control participants to enhance study design), which raises the potential for bias.

Though there were no significant differences in age, height, weight, or pKAM at the pre time point, the pre- to post-aNMT functional connectivity changes and their relation to biomechanical adaptations warrants cautious interpretation due to the present study's experimental design. Future studies should consider comparing standard NMT to aNMT and/or integrating complementary 'sham' conditions (Bonnette, DiCesare, Kiefer, et al., 2019) with larger sample sizes and group randomization to further understand the effect of aNMT on functional connectivity. Further, as superior dose-response relationships are critical for ACL prevention effectiveness (Sugimoto, Myer, Bush, et al., 2012), future large-scale studies should use compliance data as covariates (and/or predictors) and consider additional neuroimaging sessions (e.g., after 2 weeks, 4 weeks, etc.) to further refine the relative effects of aNMT on biomechanics and functional connectivity.

4.2 Conclusion

These data provide preliminary evidence of potential neuroplastic mechanisms underlying changed movement mechanics resulting from NMT, supplemented with real-time, interactive, visual biofeedback. Not only do these data reveal how the brain may be adapting in response to aNMT, but they may help guide researchers who have called for a paradigm shift toward 'brain-training' in musculoskeletal rehabilitation (Armijo-Olivo, 2017). In addition to ACL injury prevention, aNMT may be useful for other painful musculoskeletal disorders (e.g., patellofemoral pain) that exhibit alterations in brain structure and function (Silfies et al., 2017). Simple biofeedback modifications (e.g. adjusting the relative gain of visual and sensory information) could potentially leverage neuroplastic mechanisms for enhanced injury prevention and rehabilitation efforts.

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Figure Captions

Figure 1: An illustration of a participant performing a double leg squat while interacting with the augmented neuromuscular training stimulus. The stimulus was mapped on to key biomechanical variables that would distort in real-time as a function of participants' movements.

Figure 2: *The percent change in peak knee abduction moment from pre to post was significantly greater (i.e., knee moment decreased) for the augmented neuromuscular training group compared to the control group ($p = .03$).

Figure 3: Regression analyses revealed that, for the participants who completed augmented neuromuscular training, greater improvement in the percent change in peak knee abduction

moment was related to increased connectivity at post between the right cerebellum (anterior division; more inferior circle) and right thalamus ($p = .0292$; false discovery rate corrected; more superior circle). Left image is an anterior view of the brain.

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Table 1: Characteristics of Study Participants at First Study Visit ('pre' time point)

Subject ID	Group	Age*	Height (cm)*	Weight (kg)*	pKAM (Nm)*
1	Control	16	171	60.2	-21.60
2	Control	16	164	60.3	-7.24
3	Control	17	168	49.7	-3.38
4	Control	16	170	57.0	-8.44
5	Control	17	163	62.4	-3.60
6	Control	16	160	51.3	-6.66
7	Control	15	169	81.6	-25.31
8	Control	16	166	68.2	-6.15
9	Control	16	159	59.4	-25.62
10	Control	17	162	50.6	-13.25
11	Control	17	157	52.2	-5.09
12	Control	15	163	64.6	-4.79
13	Control	17	158	56.8	-13.60
14	aNMT	16	173	63.9	0.97
15	aNMT	16	169	53.6	-15.37
16	aNMT	16	171	58.6	-16.33
17	aNMT	15	168	68.7	-33.11
18	aNMT	17	170	58.5	-21.43
19	aNMT	15	174	54.5	-13.87
20	aNMT	17	170	52.9	-32.26
21	aNMT	16	166	57.6	-22.20
22	aNMT	15	165	66.8	-17.81
23	aNMT	14	155	45.4	-12.43
24	aNMT	16	168	59.9	-14.24
25	aNMT	14	151	40.9	-17.74
26	aNMT	15	158	43.6	-19.02
27	aNMT	15	162	49.8	-6.49
28	aNMT	17	161	57.3	-1.48
29	aNMT	16	167	73.8	-40.52
30	aNMT	17	169	87.9	-34.43

aNMT: augmented neuromuscular training

pKAM: peak knee abduction movement (bilateral average of three trials)

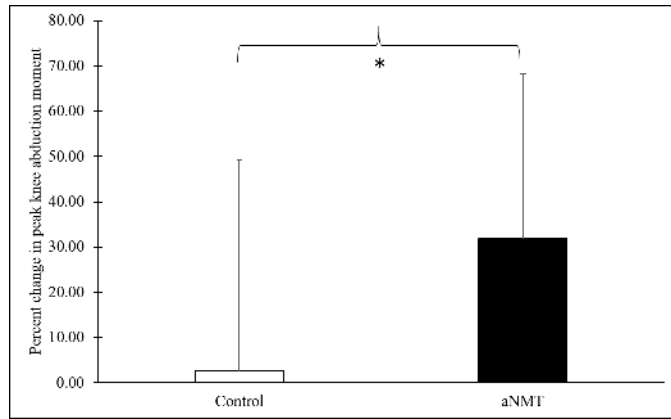
*No significant between-group differences (all $p > .05$; two-tailed)

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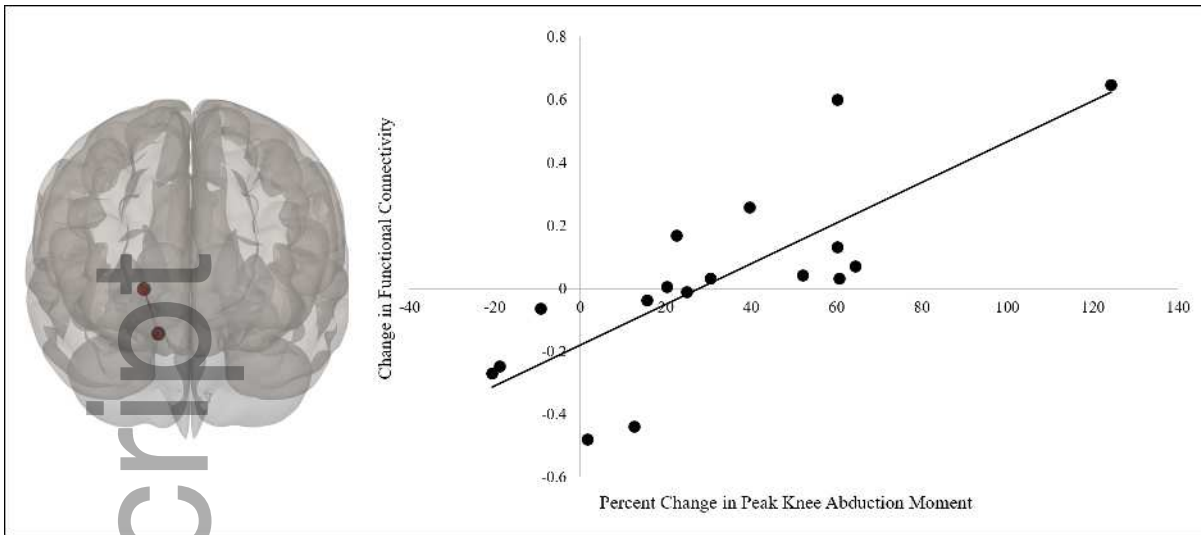


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