Sex differences in brain correlates of STEM anxiety

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ABSTRACT

Anxiety is known to dysregulate the salience, default mode, and central executive networks of the human brain, yet this phenomenon has not been fully explored across the STEM learning experience, where anxiety can impact negatively academic performance. Here, we evaluated anxiety and large-scale brain connectivity in 101 undergraduate physics students. We found sex differences in STEM-related but not clinical anxiety, with longitudinal increases in science anxiety observed for both female and male students. Sex-specific impacts of STEM anxiety on brain connectivity emerged, with male students exhibiting distinct inter-network connectivity for STEM and clinical anxiety and female students demonstrating no significant within-sex correlations. Anxiety was negatively correlated with academic performance in sex-specific ways at both pre- and post-instruction. Moreover, math anxiety in male students mediated the relation between default mode-salience connectivity and course grade. Together, these results reveal complex sex differences in the neural mechanisms driving how anxiety impacts STEM learning.

Today's universities and colleges are tasked with the challenge of developing novel strategies for improving undergraduate academic performance and ensuring that students are prepared for successful careers. In particular, emphasis is placed on enhancing student outcomes and generating enthusiasm for the science, technology, engineering, and mathematics (STEM) disciplines. However, STEM students encounter unique challenges given the time-consuming and intensive coursework. As such, many students often struggle with STEM-related anxiety, which manifests as an unease, avoidance, or fear of learning science or math topics. STEM anxiety can inhibit performance in classroom settings and increase avoidance of STEM classes altogether[1-3]. In particular, female students are presented with numerous STEM-related barriers that adversely impact achievement and performance[4,5], including stereotype threat[6], gender-based bias[7,8] and lack of non-stereotypical role models[9,10]. As a result, female STEM students, relative to their male counterparts, are disproportionately affected by higher rates of STEM anxiety[11-17].

Despite the wealth of literature regarding STEM anxiety, little work has characterized the large-scale brain networks that may be linked with this barrier to learning and achievement. However, significant prior neuroimaging research has contributed to our understanding of the neurobiological substrates of clinical anxiety and related psychiatric disorders (for reviews see: e.g., Shin *et al.*[18]; Etkin *et al.*[19]; Peterson *et al.*[20]; Mochcovitch *et al.*[21]; Duval *et al.*[22]; Williams *et al.*[23]; Kim *et al.*[24]). In the context of psychopathology, a relatively recent paradigm shift from functional localization studies to large-scale brain network studies has occurred. Psychopathological processes, especially those found in mood disorders, are associated with aberrant organization and functioning of three key networks. First, the salience network (SN), anchored in the dorsal anterior cingulate cortex and frontoinsular cortex, plays a critical role in saliency detection, and attentional capture[25,26]. Second, the default mode network (DMN), which includes the major nodes of the posterior cingulate and medial prefrontal cortices, is involved in self-referential processes and typically deactivates during stimulus-driven cognitive tasks[27-29]. Third, the central executive network (CEN) is a frontoparietal system that includes the dorsolateral prefrontal and lateral posterior parietal cortices and is involved with cognitive processes such as working memory, problem solving, and goal-directed behavior[25,30]. The interactions of these three large-scale networks underlies a unifying tripartite network model that seeks to characterize the maladaptive network organization and function common across psychiatric disorders[31,32]. Within anxiety-related disorders, increased interactions between the SN, DMN, and CEN have been consistently observed[33-36] and SN-CEN and DMN-SN interactions have been associated with trait anxiety in obsessive compulsive disorder[35] and diagnostic status in social anxiety disorder[36].

Here, we sought to bridge these two research domains by examining how STEM anxiety impacts brain activity, with an emphasis on how functional connectivity between the SN, DMN, and CEN may differ among female and male students. To this end, we collected self-report questionnaire and neuroimaging data from 101 university students (46F, 55M) enrolled in an introductory physics course. Introductory physics is a STEM gateway course that is challenging for many students. Students completed behavioral and resting state functional magnetic resonance imaging (rs-fMRI) sessions at the beginning (pre-instruction) and ending (post-instruction) of the course. Science, spatial, and math anxiety questionnaires were completed to collectively assess STEM-related anxiety[11,37,38]. In addition, the Beck anxiety inventory was completed to assess clinical anxiety symptoms[39]. To examine the relationships among STEM anxiety, brain connectivity, and sex, we addressed the following fundamental questions. First, are there sex differences in anxiety scores? Second, is there a relationship between STEM and clinical anxiety and functional connectivity? Third, are anxiety scores correlated with academic performance? Finally, does anxiety mediate the relationship between functional connectivity and academic performance? We predicted that anxiety scores would be significantly different for female versus male students. We also

anticipated that functional connectivity would be correlated with STEM anxiety among both females and males, particularly when considering the SN. Finally, we hypothesized that STEM anxiety would be negatively correlated with academic performance for both female and male students.

RESULTS

Sex differences in STEM anxiety. Science, spatial, and math anxiety were significantly higher in female compared to male students at the beginning of the semester (pre-instruction: Mann-Whitney $U_{science}$ = 944.5, P = 0.028, *Cohen's d (d)* = 0.446; $U_{spatial}$ = 918.5, P = 0.018, d = 0.484; U_{math} = 788.5, P = 0.001, d = 0.683), as well as after the course concluded (post-instruction: $U_{science}$ = 863.5, P = 0.006, d = 0.566; $U_{spatial}$ = 794.5, P = 0.001, d = 0.674; U_{math} = 894.5, P = 0.011, d = 0.519); observed P values are reported for all statistical comparisons deemed significant after controlling for a false discovery rate of 0.25 using the Benjamini-Hochberg Procedure[40] (**Fig. 1**). No significant sex differences were observed for clinical anxiety at either time point (pre-instruction: $U_{clinical}$ = 1032.0, P = 0.111, d = 0.320; post-instruction: $U_{clinical}$ = 999.5, P = 0.069, df = 0.366). When considering how students' anxiety changed across the semester-long course, only science anxiety was observed to differ across time. For female students, science anxiety scores were significantly increased at post-instruction (M = 16.43, SD = 10.76) compared to pre-instruction (M = 6.41, SD = 7.96) (t(45) = -6.63, P < 0.001, d = 1.06). Similar results were observed for male students: science anxiety scores were significantly increased at post-instruction (M = 11.28, SD = 9.563) compared to pre-instruction (M = 3.15, SD = 3.498) (t(55) = -7.671, P < 0.001, d = 1.13). However, a mixed ANOVA indicated that there was no significant interaction between participant sex and change in anxiety scores ($F_{science}$(1, 99) = 0.035, P = 0.852, η_p^2 = 0.00; $F_{spatial}$(1, 99) = 0.326, P = 0.569, η_p^2 = 0.003; F_{math}(1, 99) = 0.994 , P = 0.321, η_p^2 = 0.10; $F_{clinical}$(1, 99) = 0. .681, P = 0 .411, η_p^2 = 0.007).

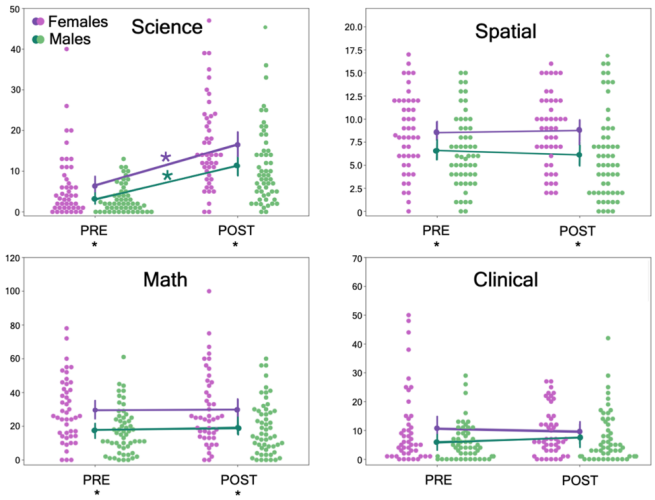

Fig. 1. Sex differences in anxiety. *Raw scores for science, spatial, math, and clinical anxiety (as measured by the Beck anxiety inventory) for female (purple) and male (green) undergraduate students enrolled in an introductory physics course. Anxiety was assessed at the beginning of the semester (i.e., pre-instruction or "PRE") and at the completion of the course (i.e., post-instruction or "POST"). Black asterisks on bottom PRE/POST labels indicate significant sex differences in anxiety at PRE or POST. Purple and green asterisks indicate significant increases in science anxiety across time.*

Neural correlates of anxiety. To assess how functional brain connectivity relates to anxiety, we first identified the SN, DMN, and CEN using a data-driven, meta-analytic parcellation[41] (**Fig. 2**), extracted the

average network time series from pre-processed rs-fMRI data, and constructed per-participant adjacency matrices reflecting the degree of between-network correlation across the three networks[42]. Motion was regressed out and high-motion volumes were censored[43]. The edge weights between the tripartite network connections were calculated as Pearson's correlation coefficients between each network time series (e.g., inter-network functional connectivity between CEN-DMN, DMN-SN, and SN-CEN).

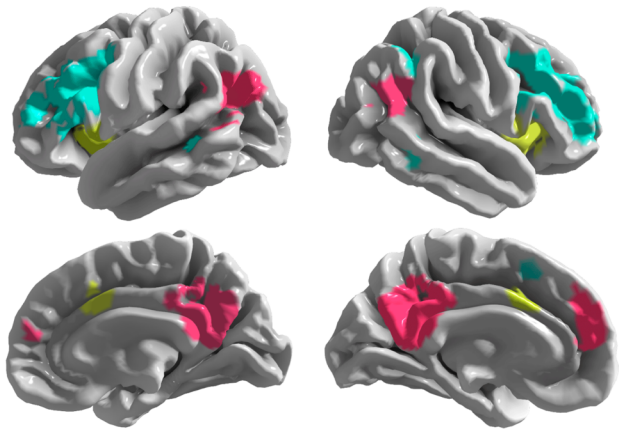

Fig. 2. Network Parcellation. *Network masks for the central executive (cyan), default mode (pink), and salience (yellow) networks were adapted from a data-driven, meta-analytic parcellation[41] and used to extra network-wise signals from pre-processed rs-fMRI data from each participant.*

To quantify putative relations between functional connectivity and anxiety, Pearson correlation coefficients were computed between the inter-network edge weights and anxiety scores, controlling for a false discovery rate of 0.25 using the Benjamini-Hochberg Procedure[40] (**Fig. 3**). At pre-instruction, among female students, there were no significant correlations between any of the anxiety scores and inter-network connectivity. In contrast, male students at pre-instruction exhibited significant correlations between science anxiety and CEN-DMN connectivity ($r(53) = 0.275$, $P = 0.042$, $\alpha_{FDR} = 0.13$), science anxiety and DMN-SN ($r = 0.311$, $P = 0.021$, $\alpha_{FDR} = 0.10$), spatial anxiety and CEN-DMN ($r = 0.366$, $P = 0.006$, $\alpha_{FDR} = 0.02$), math anxiety and CEN-DMN ($r = 0.325$, $P = 0.015$, $\alpha_{FDR} = 0.08$), math anxiety and DMN-SN ($r = 0.355$, $P = 0.008$, $\alpha_{FDR} = 0.04$), and clinical anxiety and SN-CEN ($r = -0.343$, $P = 0.010$, $\alpha_{FDR} = 0.06$). The correlation between clinical anxiety and SN-CEN connectivity was the only significant negative correlation observed, as well as the only measure linked with SN-CEN connectivity. All STEM anxiety measures in males were positively correlated with the CEN-DMN and DMN-SN connectivity. We also tested for an effect of sex across these results and observed that the correlation between clinical anxiety and SN-CEN was significantly different between female and male students ($Z = -2.927$, $P = 0.002$).

At post-instruction, no significant correlations were observed between anxiety scores and inter-network connectivity for female students. Male students at post-instruction exhibited significant correlations between spatial anxiety and CEN-DMN ($r(53) = 0.381$, $P = 0.004$, $\alpha_{FDR} = 0.04$), spatial anxiety and DMN-SN ($r = 0.435$, $P = 0.001$, $\alpha_{FDR} = 0.02$), and math anxiety and DMN-SN ($r = 0.332$, $P = 0.013$, $\alpha_{FDR} = 0.06$). As with pre-instruction results, the significant STEM-related correlations were positive and only impacted the CEN-DMN and DMN-SN, but not SN-CEN connectivity. Again, we also tested for an effect of sex across these results and observed that the spatial anxiety correlations with CEN-DMN and DMN-SN and significantly differed between female and male students ($Z = -2.375$, $P = 0.009$ and $Z = 3.094$, $P = 0.001$, respectively).

In addition, we examined the correlations between the change in anxiety scores and the change in connectivity from pre- to post-instruction. Of these, $\Delta anxiety_{spatial}$ and ΔSN-CEN were significantly negatively correlated for females ($r(44) = -0.459$, $P = 0.001$, $\alpha_{FDR} = 0.02$), but not males $r(53) = -0.041$, $P = 0.764$, $\alpha_{FDR} = 0.23$), and the difference between sexes was statistically significant, $Z = 2.208$, $P = 0.014$. Thus, for female students, as spatial anxiety increased, connectivity between SN and CEN decreased. Conversely, $\Delta anxiety_{math}$ and ΔSN-CEN were significantly negatively correlated among male students ($r(53) = -0.361$, $P = 0.007$, $\alpha_{FDR} = 0.02$), but not female students $r(44) = -0.057$, $P = 0.707$, $\alpha_{FDR} = 0.17$), and this difference between sexes was statistically significant, $Z = -1.557$, $P = 0.06$. Thus, for male students, as math anxiety increased, connectivity between the SN and CEN decreased.

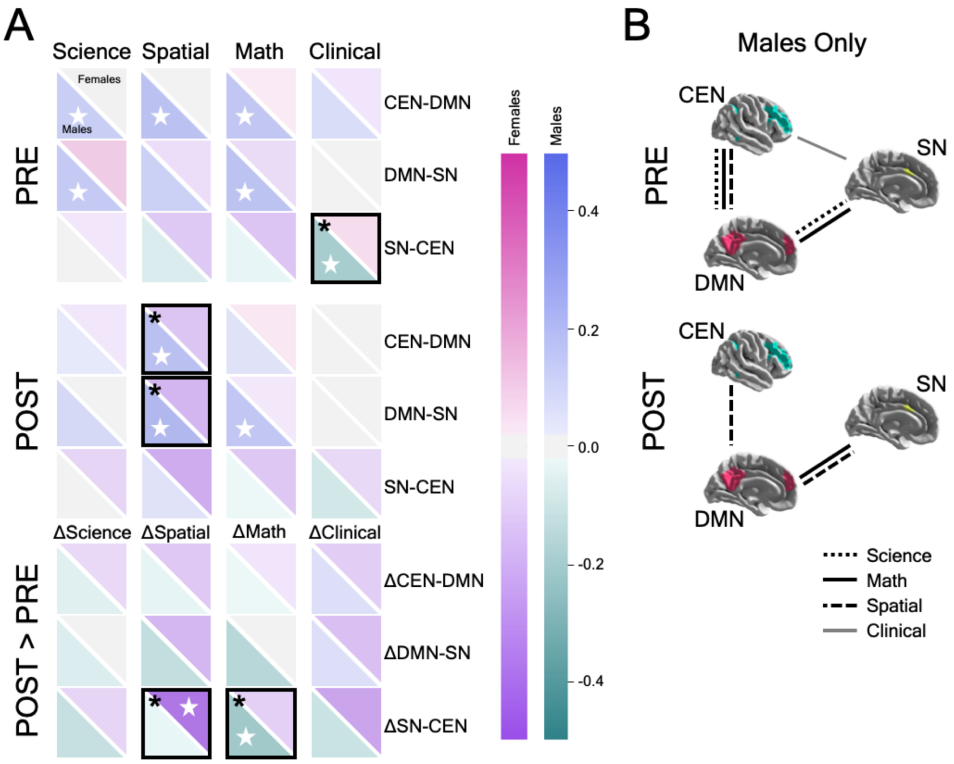

Fig. 3. Anxiety and functional brain connectivity. *(A) Correlation values are shown between science, spatial, math, and clinical anxiety (columns) and between-network tripartite connectivity between the SN, DMN, and CEN networks (rows). Correlations are displayed for pre-instruction ("PRE"), post-instruction ("POST"), and the change across time ("POST > PRE"). Each square represents the correlation between anxiety and inter-network connectivity, with the upper diagonal displaying the value for female students and the lower diagonal representing male students. Positive and negative correlations are indicated by the color bars. Significant within-sex correlations are indicated by a white star, while significant between-sex correlations are indicated by a black box with an asterisk. (B) An alternative visualization of the results is provided to delineate the between-network correlations with anxiety in male students. While female students exhibited no significant correlations between anxiety and brain connectivity at pre- or post-instruction, male students exhibited several significant correlations at both time points. Males exhibited a general tendency to show fewer significant correlations at post- compared to pre-instruction impacting a reduced set of tripartite connections*.

Sex, anxiety, and academic performance. Traditional measures of academic performance include measures of students' grades. We collected each student's overall GPA prior to taking the course, as well as their final physics course grade. First year students were excluded (2F, 6M) from the GPA analysis since

they entered the physics course with a GPA of zero. No significant sex differences were observed for incoming GPA (U_{GPA} = 1051.5, P = 0.838, d = 0.293) or physics course grade (U_{grade} = 1056.5, P = 0.148, d = 0.286).

To quantify the relation between anxiety and academic performance, Pearson correlations were computed separately for female and male students, controlling for a false discovery rate of 0.25 using the Benjamini-Hochberg Procedure[40] (**Fig. 4**). Among female students at pre-instruction, GPA was positively correlated with spatial anxiety ($r(42)$ = 0.381, P = 0.011, α_{FDR} = 0.06) while course grade was negatively correlated with math anxiety ($r(44)$ = -0.321, P = 0.030, α_{FDR} = 0.09) and clinical anxiety ($r(44)$ = -0.534, P < 0.001, α_{FDR} = 0.03). Among male students at pre-instruction, GPA was only negatively correlated with math anxiety ($r(47)$ = -0.358, P = 0.012, α_{FDR} = 0.03). The correlation between GPA and clinical anxiety at pre-instruction significantly differed between females and males (Z = 2.364, P = 0.009). Among female students at post-instruction, GPA was negatively correlated with clinical anxiety ($r(42)$ = -0.315, P = 0.037, α_{FDR} = 0.06), and grade was negatively correlated with both math anxiety ($r(44)$ = -0.293, P = 0.048, α_{FDR} = 0.09) and clinical anxiety ($r(44)$ = -0.401, P = 0.006, α_{FDR} = 0.03). Among male students at post-instruction, GPA was negatively correlated with science anxiety ($r(47)$ = -0.370, P = 0.009, α_{FDR} = 0.09) and math anxiety ($r(47)$ = -0.449, P = 0.001, α_{FDR} = 0.03), and similarly, grade was also negatively correlated with science anxiety ($r(53)$ = -0.354, P = 0.008, α_{FDR} = 0.06) and math anxiety ($r(53)$ = -0.422, P = 0.001, α_{FDR} = 0.03). Thus, in general, high levels of post-instruction STEM anxiety were associated with poor academic performance. No significant sex differences at post-instruction were observed.

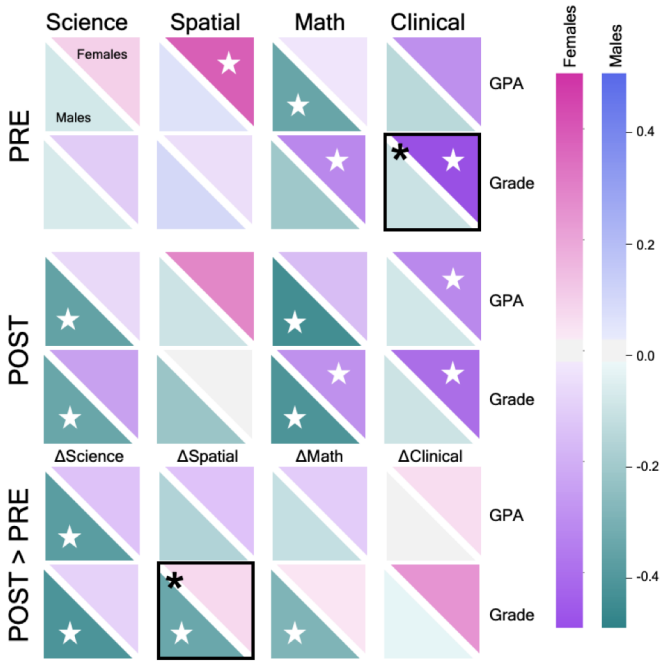

Fig. 4. Sex, anxiety, and performance. *Correlation values are shown between science, spatial, math, and clinical anxiety (columns) and pre-semester GPA and physics course grade (rows). Correlations are provided for pre-instruction ("PRE"), post-instruction ("POST"), and the change across time ("POST > PRE"). Each square represents the correlation between anxiety and GPA/grade, with the upper diagonal displaying the value for female students and the lower diagonal representing the male students. Positive and negative correlations are indicated by the color bars. Significant within-sex correlations are indicated by a white star, while significant between-sex correlations are indicated by a black box with an asterisk.*

Next, we examined the correlations between the change in anxiety scores and academic performance. Female students demonstrated no significant correlations between GPA or grade and the change in any anxiety measure. Conversely, male students exhibited significant negative correlations between grade and $\Delta anxiety_{science}$ ($r(53) = -0.393$, $P = 0.003$, $\alpha_{FDR} = 0.03$), $\Delta anxiety_{spatial}$ ($r = -0.339$, $P = 0.011$, $\alpha_{FDR} = 0.06$), and $\Delta anxiety_{math}$ ($r = -0.296$, $P = 0.028$, $\alpha_{FDR} = 0.09$), as well as between GPA and $\Delta anxiety_{science}$ ($r(47) = -0.416$, $P = 0.003$, $\alpha_{FDR} = 0.03$). A significant sex effect was observed for the correlation between grade and $\Delta anxiety_{spatial}$ ($Z = -2.033$, $P = 0.021$).

Anxiety mediates brain function and performance. Lastly, we investigated if functional brain connectivity was correlated with academic performance at pre- or post-instruction, controlling for a false discovery rate of 0.25 using the Benjamini-Hochberg Procedure[40] (**Fig. 5a**). For female students, no significant correlations were observed between inter-network brain correlations and GPA or course grade at either time point. For male students, there was a significant, negative correlation between DMN-SN connectivity and course grade at post-instruction ($r(53) = -0.267$, $P = 0.049$, $\alpha_{FDR} = 0.09$). Given this result, we then asked to what extent anxiety might mediate the relationship between brain connectivity and academic performance. We investigated four separate mediation models among male students to determine if post-instruction science, spatial, math, or clinical anxiety was a mediating variable on DMN-SN connectivity and course grade. We observed including math anxiety as a variable reduced the total effect of DMN-SN and course grade, which was no longer significant (*indirect effect* = -0.544, *SE* = 0.267, *P* = 0.042; *95% confidence intervals* (*CIs*) = -1.161, -0.128) (**Fig. 5b**). Science, spatial, and clinical anxiety were not found to mediate DMN-SN connectivity and course grade.

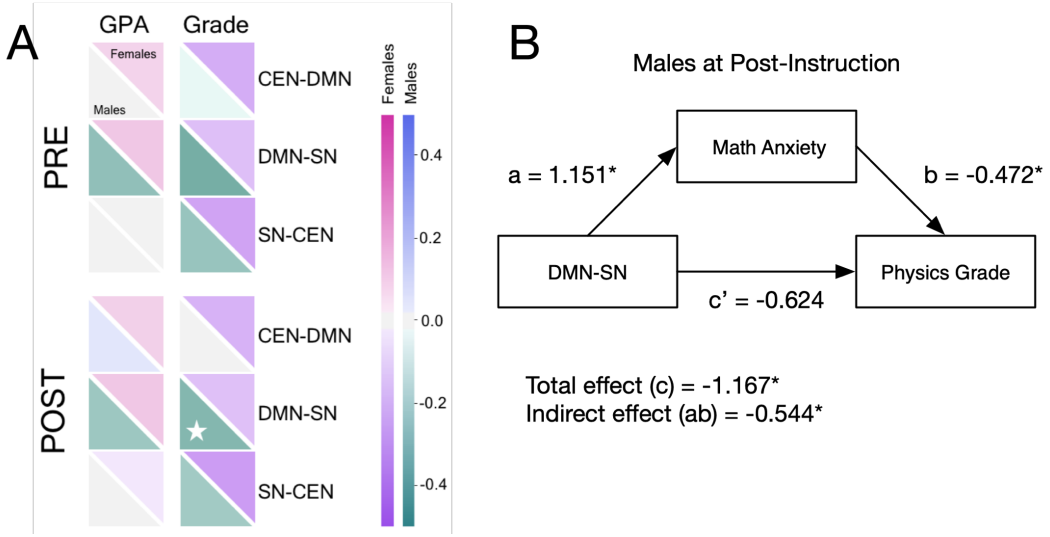

Fig. 5. Post-instruction math anxiety mediates the relation between DMN-SN connectivity and physics course grade. *(A) Correlation values are shown between pre-semester GPA and physics course grade (columns) and between-network tripartite connectivity between the SN, DMN, and CEN networks (rows). Correlations are provided for pre-instruction ("PRE") and post-instruction ("POST"). Each square represents the correlation between GPA/grade and inter-network connectivity, with the upper diagonal displaying the value for female students and the lower diagonal representing the male students. Positive and negative correlations are indicated by the color bars. Significant within-sex correlations are indicated by a white star. (B) Results of the mediation analysis indicated that every 1-unit increase in post-instruction DMN-SN connectivity was associated with a a = 1.151 (SE = 0.427, P = 0.007) unit increase in post-instruction math anxiety. Adjusting for post-instruction DMN-SN connectivity, every unit increase in post-instruction math anxiety was associated with a b = -0.472 (SE = 0.144, P = 0.001) unit decrease in course grade. Increases in post-*

instruction DMN-SN connectivity were associated with decreases in course grade, indirectly through increases in post-instruction math anxiety. Specifically, for every a = 1.151-unit increase in post-instruction math anxiety, there was a ab = -0.544 (SE = 0.267, P = 0.042) unit decrease in course grade. Importantly, a bias-corrected bootstrapped confidence interval with 10,000 samples[44] did not contain 0, 95% CI [-1.161, -0.128], indicating a significant indirect effect (ab). Last, there was no sufficient evidence that post-instruction DMN-SN connectivity was significantly associated with course grade, independent of its association with post-instruction math anxiety, c' = -0.624 (SE = 0.624, P = 0.318).

DISCUSSION

Our results identified significant sex differences in STEM, but not clinical anxiety, among undergraduate physics students, with females experiencing higher levels of STEM anxiety compared to their male counterparts, in agreement with prior work[11,37,38]. While we observed significantly increased science anxiety from pre- to post-instruction in both female and male students, we found no evidence of an interaction between sex and change in anxiety scores. That is, our results do not suggest that the introductory physics course in our study differentially impacts changes in anxiety for female and male students. This is important from the perspective of educators who seek to create inclusive classrooms that are free from instructionally derived bias.

Previous studies have shown that the SN, DMN, and CEN are impacted by clinical anxiety[19,33-35,45,46], including trait anxiety in a non-clinical adolescent sample[47]. We were surprised to see that female students exhibited no significant correlations between functional brain connectivity and anxiety at either time point. In contrast, male students exhibited multiple significant correlations between functional connectivity and anxiety at both pre- and post-instruction, suggesting anxiety-related disruption of inter-network equilibrium[33] between the SN, DMN, and CEN. For male students with high anxiety, strong inter-network connectivity was observed between the CEN and DMN and between DMN and SN, in agreement with previous studies[33-35]. These results provide additional STEM-relevant support for the importance of suppressing self-referential cognition[48] and identifying salient, task-relevant stimuli that should be relayed to the CEN[49-51]. DMN-SN connectivity was negatively correlated with course grade in male students at post-instruction, further supporting the importance of toggling off internal processing when salient events are detected in the context of STEM learning.

Male students exhibited a general trend of fewer significant brain-anxiety correlations at post- compared to pre-instruction, despite increased science anxiety. Although speculative, this tendency is suggestive of a cognitive or physiological mechanism at play and may provide directions for future work. As male students are faced with the challenges of their first university-level physics course, the brain may accommodate the increases in science anxiety and balance the response to such challenges. In contrast, female students experience greater obstacles in STEM education[5,6,52] that can trigger anxiety as early as the preschool and elementary years[53-55]. The null female results may point to a lack of vulnerability, suggesting that their relatively higher STEM anxiety does not hinder salience-related central executive and self-referential processes. Female students may experience an earlier adaptive period as their STEM anxiety increases, resulting in a compensatory mechanism that down-regulates the anxiety-brain correlations, possibly via a reallocation of neural resources or a functional reorganization of anxiety-related systems. Overall, it is unclear if the sex differences in functional connectivity observed here reflect experiential differences in STEM anxiety-related developmental trajectories due to disruptions in emotion regulation[56], attentional control[57-59], motivation and drive[60-62], disengagement and avoidance[63], coping strategy[64] or a combination of these influences. Further work is needed to investigate sex differences in

developmental STEM trajectories, to determine if female students experience STEM-related anxiety and learn strategies for counterbalancing their anxiety at an earlier educational stage.

Aberrant connectivity between the CEN and SN in anxious individuals may result from a diminished ability to exert cognitive control and regulate emotional responses[26]. Previous work has shown that university students with high math anxiety exhibit increased SN activity when anticipating a math problem[65], yet math cue-related activity increased in the CEN as math deficit decreased, suggesting that increased recruitment of cognitive control processes may improve performance in math[66]. Relatedly, lower math anxious children showed increased activation in regions of the CEN and DMN during math problem solving compared to higher math anxious children[67] although the reverse was shown by Supekar *et al*.[68] during successful math trials. This prior work in task-based fMRI has not addressed sex-related differences in the neural correlates of anxiety. Here, we showed math anxiety was consistently related to brain connectivity and performance for both sexes compared to other anxiety measures. Specifically, although math anxiety did not significantly impact SN-CEN inter-network connectivity in male students at pre- or post-instruction, the change in math anxiety was negatively correlated with the change in SN-CEN connectivity over the course of instruction. That is, as math anxiety increased across the semester for male students, SN-CEN connectivity also increased. Although higher levels of math anxiety are reported by female students, math anxiety has been more strongly linked to poor performance in precollege male students[69]. Our results related to math anxiety in male students suggest that the SN-CEN pathway may play a critical role in longitudinal changes across a semester of STEM learning, but that the DMN-SN pathway is more strongly related to course performance, with math anxiety mediating this relationship.

Our study is limited by several concerns. First, students diagnosed with psychiatric or neurologic disorders were excluded; participants were also excluded if they reported use of psychotropic medications. Thus, our results may not generalize to a broader community of students that includes those diagnosed with and receiving treatment for clinical disorders of anxiety and depression. Second, although our primary analyses treated STEM and clinical anxiety as independent constructs, we acknowledge that this may not be the case for some students. As such, we conducted additional analyses via partial Pearson correlations that produced approximately equal, and even in some instances stronger, associations between STEM anxiety, functional connectivity, and academic performance when controlling for clinical anxiety (Supplemental Information). Third, the timeline of the study created logistic challenges in that all data collection was carried out during short periods of time at the beginning and ending of each semester. As a result, while MRI sessions were completed following the final exam, our post-instruction behavioral data were generally scheduled the week prior to finals week (a period of time generally associated with increased anxiety levels among students). It is unclear how our results may be confounded by the temporal mismatch of MRI and behavioral sessions. Fourth, additional clarity may have been provided by including additional measures (e.g., the Positive and Negative Affect Schedule) to assess participant mood states on the day of scanning. Last, anxiety was assessed exclusively via self-report rating scales. Future work should include additional multi-method designs such as task-based fMRI with concurrent psychophysiological indexes of sympathetic and parasympathetic activity (e.g., respiratory sinus arrhythmia and skin conductance, respectively).

Overall, our results indicate that female and male students experience different levels of STEM anxiety and exhibit different neurobiological systems-level support for this anxiety, which differentially impacts their academic success. That this occurs despite no sex differences in performance (e.g., GPA or course grade) is notable, and in agreement with two recent meta-analyses[70,71] that provide strong evidence disproving the persistent stereotypes that male students outperform female students in math and science. Importantly, the course studied here was shown to be *equal* (i.e., did not adversely impact female

anxiety more than male anxiety), but not *equitable* (i.e., did not reduce sex differences). The gender gap in STEM remains largely unexplained[72], yet our results suggest that female students maintain performance compared to their male counterparts while responding differently to obstacles and challenges associated with STEM learning. Organizations supporting women in STEM have long promoted the idea that reduced female representation in STEM is due to poor climate for women rather than lack of ability or interest. Our results support this framework. We recommend that positive changes in favor of promoting women in STEM should focus on addressing climate issues that contribute to STEM anxiety. At the elementary and secondary school level this could include improving parental and teacher support, which has been shown to significantly impact girls' anxiety, confidence, and performance[53,73,74]. At the university level, this could include increasing visible role models (e.g., women as STEM faculty and in senior leadership positions[75]), revising ineffective Title IX policies, and enacting a zero-tolerance policy for sexual harassment and abuse at institutions, research societies, and federal funding agencies. It is incumbent upon university leaders to optimize pathways for all students entering the national STEM workforce. Instructional techniques focused on helping students learn content while building positive affect may be of particular importance in supporting learning that is inclusive for all students, thereby retaining individuals that drop out of STEM careers due to these climate-related factors. Continued development of instructional practices should emphasize the important distinction between equality and equity.

Broadly, female and male STEM students experience different learning environments, societal expectations, and academic opportunities, which all contribute to socio-emotional brain development, necessitating rigorous and objective standards for the study of sex and gender in neuroimaging research[76]. Our results demonstrate that sex differences in brain networks are not fixed and that STEM anxiety has an impact on shaping both female and male students' brains during the physics learning process. We conclude that there are significant sex differences between STEM anxiety linked with large-scale brain networks and recommend future research to determine how reducing barriers and making the climate more equitable may enable a more inclusive STEM community.

METHODS

Participants and Study Design. One hundred and one healthy right-handed undergraduate students (mean age = 19.94 $\pm$ 2.46 years, range = 18-25 years; 48 females) who completed a semester of introductory calculus-based physics at Florida International University (FIU) took part in this study. Participants self-reported that they were free from cognitive impairments, neurological and psychiatric conditions, and did not use psychotropic medications. The physics course emphasized problem solving skill development and covered topics in classical Newtonian mechanics, including motion along straight lines and in two and three dimensions, Newton's laws of motion, work and energy, momentum and collisions, and rotational dynamics. Students completed a behavioral and MRI session at two time points at the beginning ("pre-instruction") and conclusion ("post-instruction") of the 15-week semester. Pre-instruction data collection sessions were generally acquired no later than the fourth week of classes. Post-instruction sessions were completed no more than two weeks after the final exam. Written informed consent was obtained in accordance with FIU's Institutional Review Board approval.

Behavioral Measures. Participants completed a series of self-report instruments during their pre- and post-instruction behavior session, including, but not limited to: the Science Anxiety Questionnaire[11], the Spatial Anxiety Scale[38], the Mathematics Anxiety Rating Scale[37], and the Beck Anxiety Inventory[39]. Participants also provided their demographic details (e.g., biological sex, age).

Missing Data. A missing value analysis indicated that less than 2% of the data were missing for each variable and these were observed to be missing completely at random (MCAR). We chose not to implement multiple imputation, expectation maximization, or regression because the data violated the assumption of multivariate normality[77]. Given the small sample size, frequency of missingness (1-2%), and lack of systematic reasons for missingness, we implemented item-level mean substitution imputation to avoid case-wise deletion of missing data[78].

fMRI Acquisition and Pre-Processing. Neuroimaging data were acquired on a GE 3T Healthcare Discovery 750W MRI scanner at the University of Miami. Resting state functional MRI (rs-fMRI) data were acquired with an interleaved gradient-echo, echo planar imaging (EPI) sequence (TR/TE = 2000/30ms, flip angle = 75°, field of view (FOV) = 220x220mm, matrix size = 64x64, voxels dimensions = 3.4×3.4×3.4mm, 42 axial oblique slices). During resting-state scans participants were instructed to remain still with their eyes closed. A T1-weighted series was also acquired using a 3D fast spoiled gradient recall brain volume (FSPGR BRAVO) sequence with 186 contiguous sagittal slices (TI = 650ms, bandwidth = 25.0kHz, flip angle = 12°, FOV = 256x256mm, and slice thickness = 1.0mm). Each participant's structural T1-weighted image was oriented to the MNI152 2mm template using AFNI's (http://afni.nimh.nih.gov/afni;[79-81]) 3dresample, then skull-stripped using the Brain Extraction Tool (BET; Smith *et al.*[82]) from FMRIB's Software Library (FSL, https://fsl.fmrib.ox.ac.uk/fsl/fslwiki; Smith *et al.*[82]; Jenkinson *et al.*[83]). Utilizing FSL's automated segmentation tool (FAST), tissue-type masks were generated to inform nuisance parameters[84]. Then, utilizing FSL's FLIRT[85,86], the middle volume of each functional run was extracted and coregistered with the corresponding T1-weighted image. Utilizing FSL's MCFLIRT with spline interpolation, motion correction aligned all volumes of each subject's rs-fMRI time series with that middle volume. To further correct for in-scanner motion effects, functional volumes unduly affected by motion were identified using fsl_motion_outliers, with a framewise displacement threshold of 0.2mm[43]. Resultant motion artifacts were removed with ICA-AROMA (https://github.com/rhr-pruim/ICA-AROMA; Pruim *et al.*[87]). Then, CSF and WM masks were transformed into functional native space, eroded by 1 and 2 voxels, respectively, and from each the mean signal was extracted and used to regress out non-neural signals in a final nuisance regression step using AFNI's 3dTproject, which detrended and normalized the rs-fMRI time series, as well. Finally, rs-fMRI images were transformed into MNI152 2mm space for further data analysis.

Network Parcellation and Brain Connectivity Analyses. Each participant's rs-fMRI data were standardized and parcellated according to the meta-analytic network components from Laird *et al.*[41]. Included in this parcellation are the salience network (SN), default mode network (DMN), and central executive network (CEN). As these networks were delineated via ICA, some overlap was present between component maps. This overlap was resolved by a combination of proportional thresholding and manual editing, performed with the Mango image analysis tool (v. 4.0.1, http://ric.uthscsa.edu/mango/; Lancaster *et al.*[88,89]); final networks are shown in **Fig. 2**. Adjacency matrices were constructed per participant using Nilearn (v. 0.3.1, http://nilearn.github.io/index.html), a Python (v 2.7.13) module, built on scikit-learn, for the statistical analysis of neuroimaging data[42,90]. For each of the three networks of interest, a single time series was computed as an average of the rs-fMRI time series from all voxels within the network, after further regressing out six motion parameters (from MCFLIRT) and censoring high-motion volumes (framewise displacement >0.2mm), as well as the immediately preceding volume and two following volumes, following recommendations from Power *et al.*[43]. Edge weights for each graph were pairwise Pearson's correlations, calculated pairwise for the three networks, which are the graph's nodes, resulting in a 3x3 network-wise correlation matrix for each subject.

Statistical Analyses. All statistical tests were computed using IBM SPSS software, R Statistical Software, and Python tools/packages including Nilearn: Machine learning for Neuroimaging in Python, pandas (Python Data Analysis Library), matplotlib, Seaborn: statistical data visualization, Statsmodels, and SciPy. Observed *P* values are reported for statistical comparisons deemed significant after controlling for a false discovery rate of 0.25 using the Benjamini-Hochberg Procedure[40].

Data Availability. A GitHub repository was created at http://github.com/nbclab/PhysicsLearning/anxiety to archive the source files for this study, including data analysis processing scripts and behavioral data. The network masks for the bilateral SN, DMN, and CEN are available via NeuroVault at https://neurovault.org/collections/4727/.

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AUTHOR CONTRIBUTIONS

ARL, EB, SMP, MTS, RWL conceived and designed the project. JEB, EIB, RO acquired behavioral and fMRI data. AG, KLB, JEB, ARL analyzed data. KLB, MCR, TS contributed scripts and pipelines. AG, KLB, JEB, ARL wrote the paper. ARL contributed to all aspects of the project.

Competing Interests. The authors declare no competing interests.

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SUPPLEMENTAL INFORMATION

Participants. The present study recruited students attending Florida International University (Miami, FL) as research participants. Eligible participants had never completed a university-level physics course, were enrolled in a semester of introductory calculus-based physics (PHY 2048), and met a minimum GPA of 2.25. Participants self-reported that they were free from cognitive impairments, had not received a medical diagnosis for a neurological or psychiatric condition, did not use psychotropic medications, and presented at the first scan with no MRI contraindications. 129 individuals were consented into the study. During the pre-instruction data acquisition period (i.e., behavioral and MRI), 11 students were removed from the study due to scheduling conflicts or ineligibility concerns, yielding pre-instruction data from 118 healthy, right-handed (0.5 or greater on the Edinburgh Handedness Inventory[1]), English-speaking undergraduate students (mean age = 20.16 ± 2.32 years, range = 18-25 years; 65 females. Following pre-instruction data collection, 3 students dropped the physics course during the semester and were removed from the study, 2 were non-responsive for post-instruction scheduling, 2 were no longer interested in participating, 2 were removed due to acquired MRI contraindications or ineligibility, and 1 student was unable to schedule their final MRI visit due to scheduling conflicts. Additionally, due to equipment malfunction, the post-instruction behavioral data from 4 participants and the post-instruction MRI data from 1 participant were lost due to data corruption. A total of 103 participants completed the study at post-instruction. Two data sets were removed from the analyses due to technical issues, yielding a final sample of 101 participants in the present study. **Fig. S1** describes the missing study data across the stages of consent, data acquisition, and analysis. The final sample yielded matched (pre- and post-instruction) fMRI data sets from 101 students (mean age = 19.93 ± 2.46 years, range = 18-25 years; 48 females).

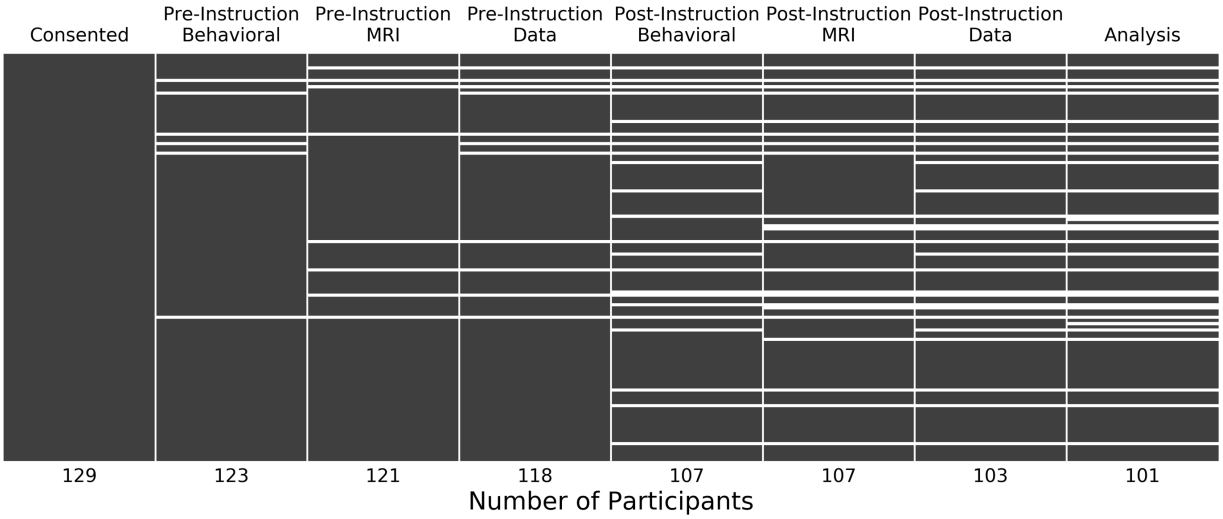

Fig. S1. Study Completeness Summary. *Display of the data table's nullity matrix for the study, describing missing data from study consent to all stages of pre- and post-instruction data collection and analysis. Visualization provided by* missingno*, a Python package for visualizing missing data (Bilogur, 2018[2]).*

The racial/ethnic demographics of the sample were 3% American Indian or Alaskan Native, 9.9% Asian, 13.9% Black or African American, 0% Native Hawaiian or Other Pacific Islander, 72.3% White, and 6.9% Other. The racial/ethnic demographics of the 65 participants who endorsed a Spanish/Hispanic/Latinx ethnicity were: 3% American Indian or Alaskan Native, 2.7% Asian, 2.7% Black or African American, 0% Native Hawaiian or Other Pacific Islander, 87.7% White, and 5.5% Other.

Study Timeline. Data were collected across six academic semesters from students in 21 separate sections of PHY 2048. Pre-instruction imaging sessions were acquired during the period starting one week prior to the initial class meeting and ending no more than four weeks into the academic semester, before the first physics course exam. Behavioral data were acquired during the same time period with the exception of two individuals who completed their pre-instruction behavioral sessions in the fifth week of the semester. In general, post-instruction behavioral data were acquired two weeks prior to the start of final exams. However, ten students were unable to complete behavioral sessions within this timeline and instead completed their sessions during or after finals week. Post-instruction imaging sessions were held after the final exam of each physics course. In most cases this meant that students underwent MRI scanning in the two weeks following the University's final exam week. However, in some cases (e.g., due to conflicts in individual's post-semester travel schedules) students were unable to complete post-instruction MRI scanning after the semester ended. Thus, a total of 15 individuals completed post-instruction MRI scanning during finals week. For these sessions the study team attempted to schedule the MRI scan after the completion of the student's full set of finals exams, resulting in 13 students who completing their post-instruction MRI scan on the last day of finals week, after all exams had concluded.

Additional Analyses: Controlling for Clinical Anxiety. Although our primary analyses treated STEM (science, spatial, and math) and clinical anxiety as independent constructs, we acknowledge that this may not be the case for some students. To assess the relationships between STEM anxiety and functional connectivity, while controlling for the effect of clinical anxiety, we conducted additional analyses of the data by computing partial Pearson correlations. Among female students at pre-instruction, when controlling for clinical anxiety, no significant partial correlations were observed between anxiety scores and inter-network connectivity. In contrast, when controlling for clinical anxiety, male students at pre-instruction exhibited significant partial correlations between science anxiety and the DMN-SN inter-network connectivity ($r(55) = 0.317$, $P = 0.020$), spatial anxiety and CEN-DMN ($r = 0.352$, $P = 0.009$), math anxiety and CEN-DMN ($r = 0.284$, $P = 0.038$), and math anxiety and DMN-SN ($r = 0.374$, $P = 0.005$). Among female students at post-instruction, when controlling for clinical anxiety, no significant partial correlations were observed between anxiety scores and inter-network connectivity. In contrast, when controlling for clinical anxiety, male students at post-instruction exhibited significant partial correlations between spatial anxiety and CEN-DMN connectivity ($r(55) = 0.383$, $P = 0.004$), spatial anxiety and DMN-SN ($r = 0.437$, $P = 0.001$), and math anxiety and DMN-SN ($r = 0.340$, $P = 0.012$). Importantly, these supplemental results are consistent with our primary results shown in **Fig. 3**, producing approximately equal, and even in some instances stronger, associations between STEM anxiety and functional connectivity in male students when controlling for clinical anxiety.

Next, we examined the correlations between anxiety scores and academic performance, controlling for clinical anxiety. Among female students at pre-instruction, when controlling for clinical anxiety, GPA was positively partially correlated with spatial anxiety ($r(44) = 0.413$, $P = 0.006$). Among male students at pre-instruction, when controlling for clinical anxiety, GPA was negatively partially correlated with math anxiety ($r(49) = -0.330$, $P = 0.022$). Among female students at post-instruction, when controlling for clinical anxiety, no significant partial correlations were observed between anxiety and academic performance. Among male students at post-instruction, when controlling for clinical anxiety, GPA was negatively partially correlated with science anxiety ($r(49) = -0.360$, $P = 0.012$) and math anxiety ($r(49) = -0.449$, $P = 0.001$), grade and science anxiety ($r(55) = -0.341$, $P = 0.012$), and grade was negatively partially correlated with math anxiety ($r(55) = -0.415$, $P = 0.002$). Again, these supplemental results are generally consistent with our primary results shown in **Fig. 4**, although some significant correlations in female students were no longer observed to be significant after controlling for clinical anxiety.

Lastly, we investigated if rs-fMRI connectivity was correlated with academic performance at pre- or post-instruction, controlling for clinical anxiety. Among female students, when controlling for clinical anxiety, no significant partial correlations were observed between inter-network brain connectivity and GPA or course grade at either time point. Among male students at post-instruction, when controlling for clinical anxiety, DMN-SN connectivity was negatively partially correlated with course grade ($r(55) = -0.296$, $P = 0.030$) and SN-CEN connectivity was negatively partially correlated with course grade ($r(55) = -0.271$, $P = 0.047$). These supplemental results are consistent with our primary results shown in **Fig. 5a** and provide added insight to the relationship between SN-CEN and academic performance. Overall, our results when controlling for clinical anxiety provide strong support for the primary results presented in this study.

SUPPLEMENTAL INFORMATION REFERENCES

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