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Measuring Sex Differences in the Corpus Callosum by Undergraduates at a Small and a Large Institution

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Neuroscience students often seem more responsive to laboratory exercises that involve human brains. Here we describe a lab that utilizes human brain MRIs to evaluate a long-standing debate over the presence of sex differences in the human brain, specifically the corpus callosum. Students at both Widener and UCLA measured corpus callosum subregions that were already marked-off as described by Witelson (1989) or by Hofer and Frahm (2006). Statistical analyses revealed sex differences using both schemes after correcting for the size of the midsagittal cortex. Widener students, however, uncovered more sex differences than the UCLA students. Lab instruction for UCLA students occurred during the COVID-19 pandemic. So, lab sessions were completely online. In contrast, Widener students had the benefit of in-person lab instruction. Nonetheless, both the data obtained from the images of the corpus callosi as well as measures of pedagogical efficacy were similar between the two institutions, suggesting that distance learning may be a valuable and viable option. Further, when in person learning is not an option, such as during a pandemic, digital databases serve as invaluable resources for online learning. When these databases are utilized in a hypothesis driven research setting, they can serve as the basis for course-based undergraduate research experiences (CUREs), which are known to benefit students—improving retention in science fields.

Key words: Online Learning; Statistical Inference; Sex Differences; Human Brain; Corpus Callosum; Big Data; Witelson; Hofer and Frahm; Subdivisions of the Brain; Biology; Neuroanatomy; Neuroscience; Psychology; Undergraduate Learning; Higher Education

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Over the past several decades, neuroscientists and psychologists have found meaningful sex differences in specific behaviors and cognitive tasks. Greater levels of aggression, rough and tumble play, as well as higher 3D rotation scores, are reported in males (Collaer and Hines, 1995), but higher verbal fluency scores are found in women (Linn and Petersen, 1985; Halpern, 1992). The overall sex differences reported in these studies are moderate, and it is well-known that there is greater variability within sex than between sexes (Joel, 2021).

Evidence for a biological link to behavioral and cognitive differences comes from research on organizational hormones, which is to say sex steroids present during early development. Organizational hormone exposure can lead to long-lasting changes in the central nervous system and behavior across vertebrates (Arnold and Gorksi, 1984; Arnold and Breedlove, 1985). Although in humans, the causal relationship of hormones and sex differences in behavior is controversial at best, some data yield anatomical sex differences in the brain. For instance, the development of the left and right sides of the hippocampus and the amygdala differ between females and males (Giedd et al., 1996), as does the ratio of grey matter to white matter (Allen et al., 2003; Gur et al., 1999). Many studies have found a sexual dimorphism of the corpus callosum (Allen et al., 1991; DeLacoste-Utamsing and Holloway, 1982; Witelson, 1989; Shiino et al., 2017), although some have not (Going and Dixson, 1990) or found conflicting results based on different methodologies (Bermudez and Zatorre, 2001).

One study finding sex differences in callosal subregions used a partitioning scheme that was based on the callosal fibers' cortical regions of origin in monkeys (Witelson, 1989). (Subsequent studies have simplified Witelson's scheme (Aboitiz et al., 1992), as depicted in Figure 1A). The rostrum fibers (Region I) are thought to extend from the inferior premotor regions and prefrontal regions (Barbas and Pandya, 1984) while more posterior fibers (Regions II, III, IV, & V) connect the motor, sensory, posterior parietal/superior temporal, and occipital cortices, respectively (Seltzer and Pandya, 1983; Cipolloni and Pandya, 1985). Witelson (1989) found a sex difference between right-handed women and men in the genu and anterior midbody (men larger than women), and in the isthmus (women larger than men), the latter of which ostensibly connects posterior parietal and superior temporal cortical regions bilaterally (Seltzer and Pandya, 1983). Her subjects, however, were postmortem cancer patients who had received different regimens during their treatment period.

Using diffusor tensor magnetic resonance imaging or DTI, Hofer and Frahm (2006) traced hemispheric fiber connectivity in the human corpus callosum based on the diffusion of water molecules. This DTI study resulted in re-mapping the corpus callosum connections (Figure 1B). Prefrontal cortex connections mapped to the anterior region of the corpus callosum (Region I), followed by premotor and supplementary motor cortex projections (Region II). The posterior regions were found to connect the motor cortices (Region III) and primary somatosensory cortex (Region IV),

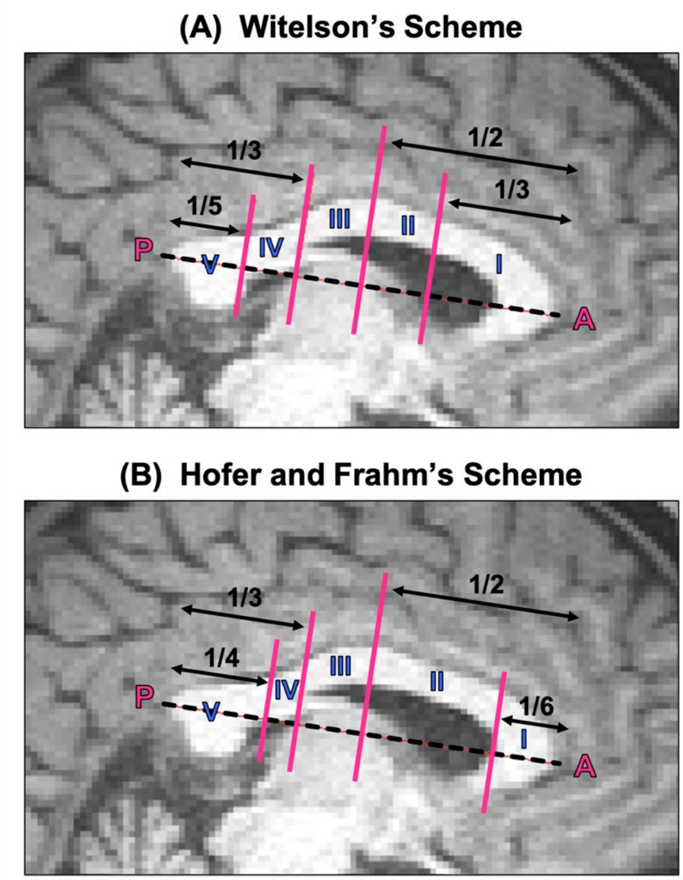

Figure 1. (A) Witelson's scheme for subdividing the corpus callosum (Witelson, 1989; as modified by Aboitiz et al., 1992). *(B)* Hofer and Frahm's proposed scheme (Hofer and Frahm, 2006). In both schemes, the vertical lines define the boundaries between subdivisions I-V, resulting in different borders for the genu-rostrum (Region I), anterior body (Region II), posterior body (Region III), isthmus (Region IV), and splenium (Region V).

with the most posterior region (Region V) forming contralateral connections to the parietal, temporal and visual cortices.

Dhaliwal and Grisham (2013) compared the Witelson and the Hofer and Frahm parcellation schemes while examining the corpus callosum for sex differences. They did not find any sex differences in the scans when using the Hofer and Frahm scheme. They did, however, find a sex difference using the Witelson approach to dividing the corpus callosum: Region IV (the isthmus) was found to be larger in men than women.

We designed a digital resource lab that allows students to test for sex differences in the corpus callosum using both the Witelson and the Hofer and Frahm schemes for subdividing the corpus callosum. We employed midsagittal magnetic resonance images (MRIs) obtained from living, healthy, human subjects. The images used are publicly accessible. In this article, we report on the methods and findings obtained by students at two different universities, Widener and UCLA, demonstrating that this lab could be successfully and accurately run in both a primarily undergraduate institution with small class sizes as well as at a larger university with larger classes. Furthermore, we show that this lab works well in both remote learning and in-person formats.

MATERIALS AND METHODS

Approval of Human Research

Prior to the start of this lab: Widener University Institutional Review Board (IRB) and the UCLA IRB approved the application to collect data on human subjects (06-19 and 18-001258, respectively).

Image Analysis

Students were presented with a background lecture on the effects of steroid hormones on sexually differentiated development and instructions on how to use the ImageJ software.

Brain midsagittal MRIs were downloaded from the OpenNeuro (https://openneuro.org/search/MRI) metasearch database. Using the drop-down menus on the web site, we selected equal numbers of healthy male and female brains, which were used at both institutions (70 brains total at Widener and 62 at UCLA) and the brains were drawn from the same set. Structural T1-weighted MRI scans orient brains in standard planes, so the midsagittal section is easy to identify–large corpus callosum with a cingulate gyrus that is mostly gray. Two individuals, who were not participating in the study, made two copies of each midsagittal section and pre-marked both with subdivisions of the corpus callosum; one copy was marked with the Witelson method, and the other was marked with the Hofer and Frahm method. For the Witelson method (Figure 1A), we measured the entire length of the corpus callosum on the image (A to P) and then divided up the subregions by the indicated fractions (e.g., one-third for the most anterior portion). For the Hofer and Frahm method, the most anterior region was one-sixth of the entire length of the corpus callosum.

Analysis of corpus callosum subregions was then conducted by undergraduate students at Widener and UCLA. Each student was assigned three to four subjects and received two brain MRI images per subject to analyze

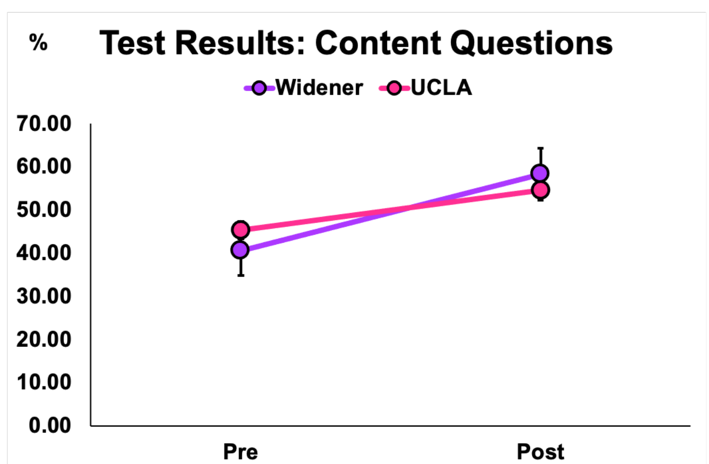

Figure 2. Pre- and post-test content question results for Widener and UCLA students.

using the ImageJ program (NIH, version 1.52a). Students were given identical instructions at both institutions. The sex of each subject analyzed was not revealed to students until the data analysis phase. Using ImageJ, students quantified the areas of the midsagittal cerebral cortex, whole corpus callosum, and five callosal sub regions on each image. These regions were traced in ImageJ using the Freehand selections tool, and the area was measured using the Analyze>Measure feature.

After all subjects' subdivision data were entered into a shared online spreadsheet, the area of each callosal sub-region was divided by either the total corpus callosum area (proportional) or the total midsagittal cerebral cortex area (ratio) to normalize the data. At UCLA, the same set of brains was used in multiple sections, so the data were averaged across student observers. No inter-rater reliability was checked. The normalized proportional and ratio data were then analyzed using repeated measures ANOVA and independent sample *t*-tests. The statistical analyses were conducted using JASP (version 0.0.9.1). After students obtained the results and engaged in group discussion, they were asked to write a lab report summarizing the background, methods, results and overall conclusions. The entire lab was run over a period of three weeks (approximately 12 hours of meeting time) at both universities.

PEDAGOGICAL RESULTS

Student Composition

Demographic data was obtained via self-report as part of the pre/post-test/questionnaire. The composition of Widener students identified as female (83%) and male (12%). Most students identified as white (67%) with the remainder identifying as Black, Asian or other race/ethnicity (33%). Eighteen Widener students took part in the exercise. All students were third- or fourth-year undergraduate biology majors taking one section of Neurobiology Lab. These students had little to moderate prior knowledge of neuroanatomy. Students at UCLA were 72% female, 28% male; 46% identified as Asian, 25% white, 16% Latinx, 2% Black, and 11% other students were primarily fourth year students. Participating UCLA students were all from a Behavioral Neuroscience Lab course, which is divided into six sections of 24 students. Since we obtained multiple measures of the same brain, we averaged them. Since this exercise was given to UCLA students during COVID, they had only been exposed to rudimentary neuroanatomy.

Pre- and Post-Test Results

We elected to assess gains directly using a pre- and post-test rather than depend on self-reporting of learning gains (e.g., Lopotto, 2007), which could be biased due to demand characteristics. At the beginning of the lab module, students were asked to fill out a consent form and, if consent was given, asked to take a pre-test to evaluate their understanding of the topic. After completion of the lab module, students took a post-test to evaluate whether the lab experience enhanced their understanding of the sex differences in the brain as well as the role of sex steroids as reflected by the corpus callosum. Students were allowed to take the post-test at their leisure.

The pre- and post-test consisted of demographic questions, 16 items that tapped content and skills from the module, and three critical thinking items. Due to the small number of items, an item analysis was not performed. Widener students demonstrated an overall increase in understanding of the material with an increase in correct responses on content items from 41% (pre-test) to 58% (post-test), $t(17) = 5.590$, $p < 0.001$, Cohen's d = 1.318 (Figure 2). UCLA students similarly demonstrated an overall increase in understanding of the material with an increase in correct responses from 45% (pre-test) to 55% (post-test), $t(111) = 5.148$, $p < 0.001$, Cohen's $d = 0.486$ (Figure 2).

Evaluation of Students within the Context of the Class

Both the UCLA and Widener students were evaluated on the basis of an assigned APA-style lab report. This report required them to read primary literature and make predictions on their outcomes based on the readings. Students had to summarize the procedure that they used to gather data. Student data were pooled and students were required (with guidance) to perform the statistical analyses and make informative graphs of the outcomes. Generally, students are unfamiliar with more complex ANOVA designs as well as the pitfall of Type I statistical error with multiple comparisons, which were both addressed. Finally, students were encouraged to discuss their outcomes as related to the

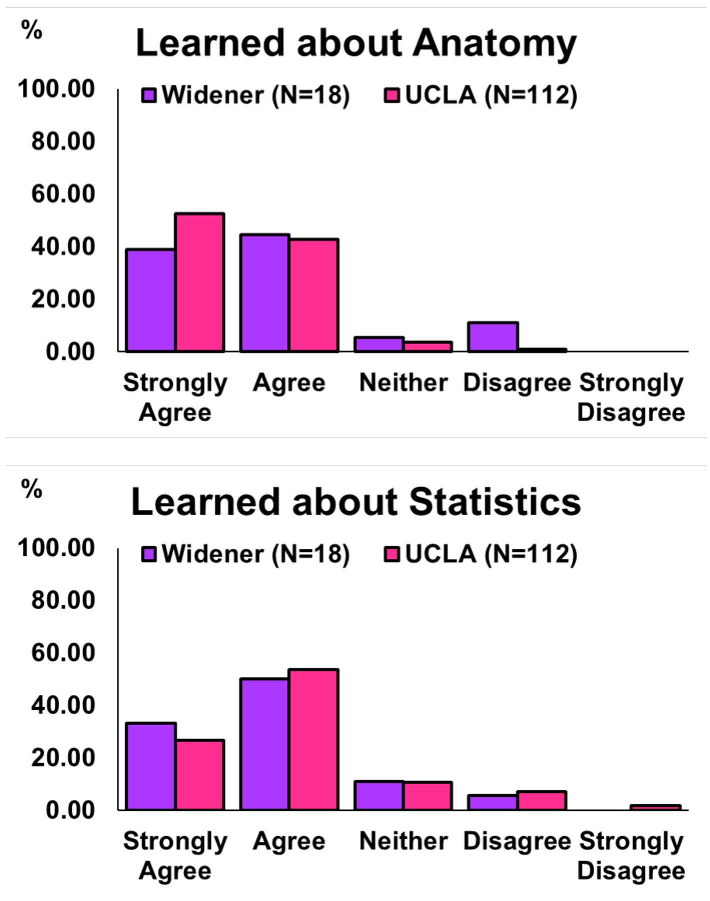

Figure 3. Widener and UCLA student responses to questions about learning callosal neuroanatomy and statistical analyses.

literature and suggest weaknesses and improvements to the study.

Student Evaluation of the Module

As a part of the post-test, students were asked to evaluate their perception of meeting specific learning objectives and their opinions about the delivery of the materials. Widener and UCLA students agreed about the value of the module in terms of learning something about corpus callosum anatomy and statistics (Figure 3). Additionally, both Widener and UCLA students were given a free response item about the purpose of the module. Again, the response pattern was highly similar (Figure 4).

Sex Difference Analysis of Corpus Callosum (*Widener Students*)

The data of two Widener students were discarded because their reported values were one-thousand-fold greater than their peers. The rest of the data of 34 male and female subjects were analyzed using corpus callosum subregion as a within-subjects variable and sex as a between-subjects variable. There was a significant sex difference, with females having a slightly larger subregion size when controlling for cerebral cortex size (ratio data) in both Witelson and Hofer and Frahm schemes ($F(1, 68) = 12.05$, $p < 0.001$, $\eta^2 = 0.012$ for Witelson's scheme; $F(1, 68) = 19.937$, $p < 0.001$, $\eta^2 = 0.025$ for Hofer and Frahm's scheme), as well as a significant sex x region interaction ($F(4, 272) = 3.708$, $p < 0.006$, $\eta^2 = 0.004$ for Witelson; $F(4, 272) = 3.008$, $p < 0.019$, $\eta^2 = 0.009$ for Hofer and Frahm). There was no effect of sex when controlling for corpus callosum overall size (proportional data).

Further statistical analyses were performed with independent *t*-tests but only with the ratio data. In the Witelson scheme, females were found to have larger subregions than males in all subregions (Table 1, Means), *p*-values ranging 0.05 to 0.001, Cohen's *d* ranging 0.0472 to 0.822. The Hofer and Frahm scheme produced a similar pattern of results (*p*-values ranging 0.01 to < 0.001; Cohen's *d* ranging 0.623 to 0.798 – Table 1, Means), except for region IV, which was not found to differ between the sexes. No sex differences were detected using raw or proportional data.

Sex Difference Analysis of Corpus Callosum (*UCLA Students*)

UCLA students obtained a pattern of results much like the Widener students. The UCLA data revealed a trend for an overall effect of sex on subregion size when controlling for cerebral cortex size (ratio data) for the Hofer and Frahm method $F(1, 60) = 3.470$, $p = 0.067$, $\eta^2 = 0.005$ with females having slightly greater subregions than males. Analyses of the Witelson scheme yielded no significant sex differences nor interaction of sex x subregion (p > .41 or more).

Similar to the Widener students, UCLA students found a somewhat different pattern of results when using independent *t*-tests; sex differences were detected in a few subregions. Using ratio data, the most anterior region of the corpus callosum was significantly larger in females than males for both the Witelson and Hofer and Frahm schemes ($t(60) = 2.153$, $p < 0.05$, Cohen's $d = 0.547$ (Table 1) for Witelson's scheme; $t(60) = 2.624$, $p < 0.05$, Cohen's $d = 0.667$ (Table 1) for Hofer and Frahm's scheme). So even though the overall ANOVAs only yielded a trend, employing multiple *t*-test revealed some significant sex differences. This pattern of data led to interesting discussions about multiple comparisons and Type I errors (see below).

DISCUSSION

Pedagogical Data

We utilized a protocol from prior studies that also examined teaching quantitative neuroanatomy (Grisham et al., 2003;

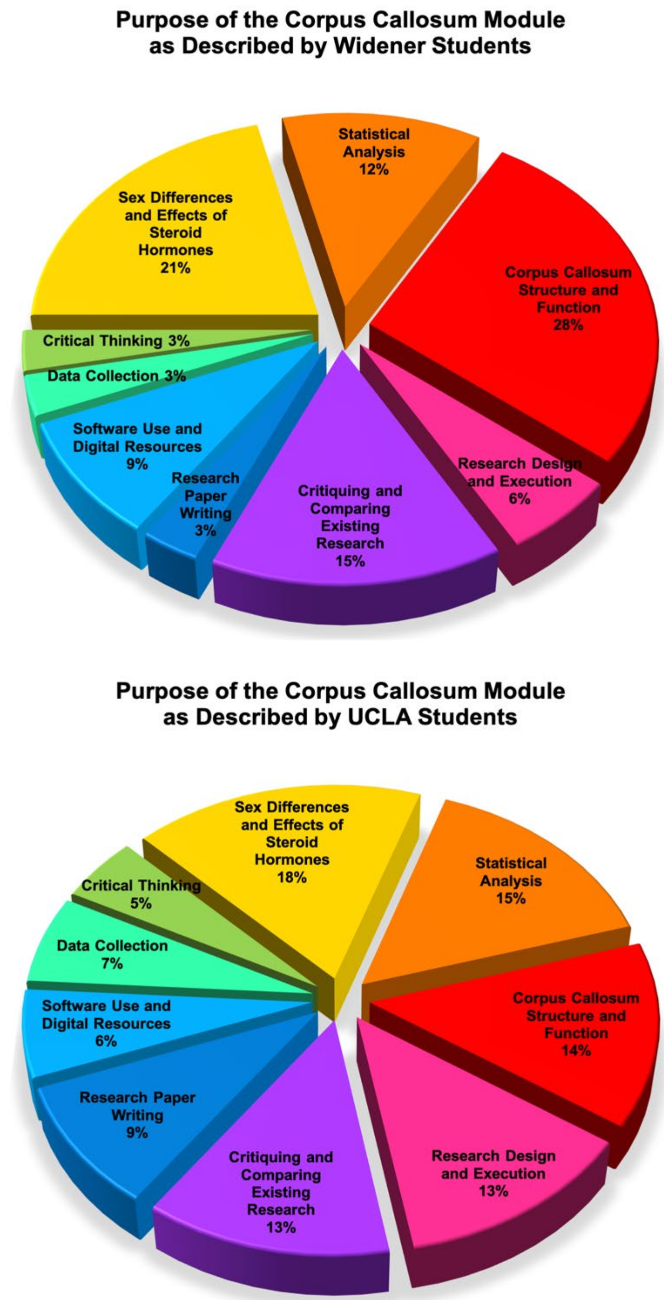

Figure 4. Student responses to the purpose of the module. A given student's response could appear in more than one category.

Scheme and Region	Widener Female Means	Widener Male Means	UCLA Female Means	UCLA Male Means
W I	0.032	0.028	0.028	0.025
W II	0.010	0.009	0.121	0.123
W III	0.010	0.008	0.009	0.008
W IV	0.008	0.007	0.007	0.007
W V	0.023	0.020	0.020	0.019
HF I	0.022*	0.017	0.018*	0.016
HF II	0.021*	0.019	0.019*	0.017
HF III	0.010*	0.008	0.008	0.008
HF IV	0.005*	0.004	0.004	0.004
HF V	0.027*	0.023	0.023	0.022

Table 1. Mean values of ratio data for callosal subregions at both Widener and UCLA and across both Witelson (W) and Hofer and Frahm (HF) parcellation schemes. An asterisk (*) indicates that a sex difference was found with an independent *t*-test.

2012; 2018) for evaluating student learning gains, their opinions, and their impressions of the module's purpose. This multi-pronged approach yielded data that were quite similar across the two institutions, including learning gains (Figure 2), affective responses/opinions/self-report (Figure 3), and perceived purpose of the module (Figure 4). All of these methods suggest that the module was an effective learning experience.

Discussing Corpus Callosum Data with Students

Several intriguing questions can be raised with students about their data analyses. At both institutions, the data were initially analyzed via ANOVAs, using sex as a between-subjects variable and subregion of the corpus callosum as a within-subjects variable. Subsequently, the data were analyzed via multiple independent *t*-tests (Table 1). This sets the stage for a lesson in statistics and the probability of committing Type I statistical errors. Some questions to consider are: Why are some results significant using one set of analyses but not another? What is the false discovery rate (Type I errors) when multiple *t*-tests are performed? Are the significant findings actually Type I errors or are the sex differences just weak effects that are somewhat obscured by utilizing an ANOVA with the callosal subregions as a within-subjects variable? Should the students' data be corrected for midsagittal brain size or corpus callosal size? Should brain size even matter in these measures (Luders et al., 2014)?

Another question to consider is whether the brain samples differ. Both the Widener and UCLA students found some evidence for a sex difference with females having a relatively larger corpus callosum in the most anterior region than males have. Nonetheless, the literature on sex differences in the corpus callosum is fraught with contradictions. Similar to our students', Shiino et al. (2017) found a sex difference (females > males) in the genu (Region I) using a volumetric approach. DeLacoste-Utamsing and Holloway (1982), in contrast, did not find a sex difference in the anterior area, but found females had a larger splenium area (Region V) than males. Allen et al. (1991) found that the splenium was shaped differently in females than males, but did not find a sex difference in area. Notably, neither the Widener nor the UCLA students replicated the results of Dhaliwal and Grisham (2013), who used yet a different sample of brains and found a sex difference in the isthmus (Region IV; males > females) with Witelson's scheme but not with the Hofer and Frahm scheme.

Both the Widener and UCLA students' findings flatly contradicted Witelson's (1989) original report: Witelson found the anterior regions of the corpus callosum to be larger in males. Witelson's data, however, were collected by measuring postmortem tissue from cancer patients. The patients she recruited had either lung or breast cancer, and these diseases, as well as the treatments they had, could have differentially affected the corpus callosum area or volume. In contrast, our students' measurements were conducted on MRI scans from healthy living subjects.

Although the pattern of the corpus callosum data obtained by students at the two institutions bore clear similarities, there were clearly some significant findings at Widener that were not obtained at UCLA (Table 1). Notably, Widener students used 71 brains from the set whereas UCLA students only utilized 62. Although a single person at each institution partitioned the corpus callosum for the students, they could have differed in their selections and UCLA data may have been more variable due to multiple observers even though the data were averaged across them.

Broader Impacts

When big databases are utilized in a hypothesis driven research setting, they can serve as the foundation for Course-based Undergraduate Research Experiences (CUREs), which are known to result in improved perception of the sciences and higher retention in STEM (Villarejo et al., 2008).

The module described here is one of the few to use a big data resource in undergraduate education, and there are very few barriers to crafting a similar one. Scans can be obtained at no cost to the end-user from such sites as OpenNeuro (https://openneuro.org/search/MRI). Such rich resources allow students to seek answers to questions even apart from those that the original investigator posed.

Different projects could certainly be entertained with such large-scale databases available. For example, there are several databases available that have longitudinal scans of human brain development (see INCF's KnowlegeSpace https://knowledge-space.org/) Also, there are images of mouse brains from many different genetic lines available at the Mouse Brain Library (https://www.mbl.org/), which could even lead to a genetic analyses of differences (Grisham et al. 2011).

Further, when in person learning is not an option, such as during a pandemic, digital databases serve as invaluable resources for online learning. Instruction of UCLA students occurred during the COVID-19 pandemic. So, lab sessions were completely online. Data from Widener students came from a time in which they had the benefit of in-person lab instruction. Nonetheless, measures of pedagogical efficacy at both institutions were similar as was the data that students obtained on the task. Clearly, distance learning can not only be viable in times of emergency but also as a means to reach broader, more diverse cohorts of students.

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