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Biopsychology Lab: COMT Genotype Associations with Vagal Tone and Frontal Alpha Asymmetry

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Course-based undergraduate research experiences (CUREs) engage students in the research process to promote active learning of complex material. We created a 5-week Biopsychology Laboratory (Biopsych) CURE that integrates concepts in genetics, neurotransmission, autonomic regulation, executive function, electro-encephalography, and human subjects research. The underlying principles of the Biopsych CURE focus on how the prefrontal cortex orchestrates cognitive control and coordinates parasympathetic activity. The rs4680 single nucleotide polymorphism (SNP) in the catechol-O-methyltransferase (COMT) gene may explain individual variability in prefrontal cortical function since the presence of the A versus G alleles directly affects neurotransmission in this region. To assess this, students in the Biopsych CURE conducted a prospective cohort study on themselves to examine whether there would be differences between rs4680 GG, AG, and AA genotypes in executive function, parasympathetic activity, and frontal alpha asymmetry (FAA). During the allotted class time, students successfully learned to collect buccal swab samples, isolate DNA, quantify DNA with a spectrophotometer, and use the iWorx data acquisition system to measure heart rate, vagal tone, and alpha and beta EEG waves. They also learned to analyze the data and wrote a research report on their findings. For their class research project, they found that the GG genotype had higher vagal tone compared to A carriers while taking the Stroop test, indicating greater parasympathetic activity. The GG genotype also showed higher FAA compared to A carriers while viewing emotional face presentations, indicating greater left cortical activity. This suggests that the GG genotype may display parasympathetic and cortical activity patterns that are generally conceded as advantageous to mental health. Students learned to graphically depict their data and wrote a research report on their findings. Overall, the Biopsych CURE enabled students to work actively with core topics in the field while conducting meaningful research and the course evaluations demonstrated high student satisfaction with CURE activities.

Key words: biopsychology; prefrontal cortex; dopamine; rs4680; vagal tone; executive function; frontal alpha asymmetry (FAA)

Course-based undergraduate research experiences (CUREs) engage many students in discovery research by enrolling all students in the course and enabling much of the research to be conducted during class time under direct supervision. This approach reaches a greater number of students, requires less outside time commitment, and prevents student self-selection into research experiences in comparison to undergraduate research assistantships in faculty laboratories (Auchincloss et al., 2014). Developing CUREs can be challenging because of the amount of time required for faculty to develop the experience and large class sizes (Spell et al., 2014). While CUREs for STEM fields such as biology are successful with the use of simple species such as C. elegans (Luth and Juo, 2023), psychology majors seeking CUREs are interested in methods involving human participants. Participant recruitment and retention is an additional challenge that comes with human research. We aimed to address these challenges by implementing a Biopsychology Laboratory (Biopsych) CURE across two sections of this course where the large number of students were both the investigators and the participants. This provided 35 students with practical research experience, while simultaneously obtaining a human sample size sufficiently powered to detect statistically significant results.

Enthusiasm for Biopsych CURE participation was promoted by having students test novel research questions (Spell et al., 2014). Biopsychological function was assessed based on the rs4680 single nucleotide polymorphism (SNP) in the catechol-O-methyltransferase (COMT) gene, with an emphasis on prefrontal cortical outcomes. The prefrontal cortex is the brain region largely responsible for executive function, exerting cognitive control to direct attention, impulse control, and behavioral flexibility (Barbas and Zikopoulos, 2007; Friedman and Robbins, 2022; Rossi et al., 2009). The rs4680 SNP in the COMT gene has been increasingly associated with cognitive, emotional, and mental health outcomes (Bruder et al., 2005, 2005; Lancaster et al., 2012; Woody et al., 2014; Taylor, 2018; Zareyan et al., 2020). The COMT enzyme degrades catecholamines (Boulton and Eisenhofer, 1998) and is responsible for over half of the dopamine degradation in the

prefrontal cortex (Käenmäki et al., 2010). The rs4680 SNP produces a G to A substitution at codon 158 in the COMT gene, which encodes valine or methionine, respectively. The valine (G) allele relates to greater COMT efficiency, correspondingly lower dopamine levels in the prefrontal cortex (Chen et al., 2004), and decreased performance on executive function tasks (Blasi et al., 2005; Bruder et al., 2005; Khanthiyong et al., 2019). The methionine (A) genotype is then associated with lower COMT activity, higher frontal cortical dopamine levels, and improved performance on executive function tasks (Blasi et al., 2005; Bruder et al., 2005; Khanthiyong et al., 2019).

In addition to coordinating executive function, the prefrontal cortex regulates autonomic parasympathetic activity by promoting activation of the vagus nerve (Hänsel and von Känel, 2008). This parasympathetic control enhances the ability of the prefrontal cortex to engage in stress appraisal and emotional processing (Hänsel and von Känel, 2008). Parasympathetic activity can be measured by heart rate variability (HRV) and vagal tone, which are the variation in time between heartbeats and variation in heart rate across a breath cycle, respectively. High HRV and vagal tone are associated with improved executive function, emotional regulation, and overall mental and physical health (Hansen et al., 2003; Porges, 2007; Thayer et al., 2009; McCraty and Shaffer, 2015; McLaughlin et al., 2015; Laborde et al., 2017; Edwards and Pinna, 2020).

Students were taught about the role of COMT in prefrontal cortical transmission and the tight communication between the prefrontal cortex and vagus nerve, then asked to hypothesize whether vagal tone may vary based on rs4680 genotype. They read a research article during the course which found that female A carriers with major depression have shown lower HRV (Woody et al., 2014) compared to GG individuals. The students first sought to determine whether GG individuals had higher HRV, and diminished executive function performance compared to A carriers. They learned to measure vagal tone as an index of HRV and were taught to administer executive function-based tests that activate the prefrontal cortex: the Stroop test (Jensen and Rohwer, 1966; Leung et al., 2000; Kane and Engle, 2003; Huang et al., 2022) and n-back test (Owen et al., 2005; Yaple et al., 2019; Yeung and Han, 2023).

Students also learned that activity in the prefrontal cortex can display hemispheric lateralization, measured by frontal alpha asymmetry (FAA) that indicates greater left cortical activity. Lessons introduced the concept that greater left cortical activity (higher FAA) can relate to more approach/reward-motivated behavior while greater right cortical activity (lower FAA) can relate to more withdrawal/punishment-motivated behavior (Carver and Harmon-Jones, 2009; Berkman and Lieberman, 2010; Briesemeister et al., 2013). Emotional processing can also vary based on COMT rs4680 genotype, as A carriers have demonstrated greater reactivity to unpleasant visual stimuli (Smolka et al., 2005) and enhanced recognition of facial expressions depicting negative emotions (Lischke et al., 2019).

COMT rs4680 genotype has been linked to FAA differences, but the evidence is mixed (Katz et al., 2015, Wacker et al., 2013). Therefore, the second goal for the students was to test the hypothesis that A carriers have higher FAA when viewing emotional faces. Students also sought to explore how genotype and FAA relate to behavioral approach and withdrawal systems that underlie personality, with the hypothesis that A carriers would be more approach-oriented alongside having higher FAA.

Biopsych CURE students conducted a prospective cohort study using themselves as participants. Across 5 weeks of their course, students provided salivary DNA samples for blinded genotype determination, reported heart rate and vagal tone values after the n-back test and Stroop test, self-reported scores of behavioral approach versus inhibition, and reported EEG measurements of FAA while viewing emotional face presentations. Students learned these techniques and wrote a research report on their findings. This Biopsych CURE sought to immerse students in an investigation to define the relationship between COMT rs4680 genotype and executive function, parasympathetic activity, emotional processing, cortical activity, and behavioral approaches. Beyond that, it gave many undergraduate students the opportunity to gain practical and meaningful research experience in the field of biopsychology in a relatively short period of time.

MATERIALS AND METHODS

Course Objectives

The five course objectives from the Biopsychology Lab syllabus were:

1) Demonstrate awareness of genetic and environmental determinants of behavior, and of the different approaches and designs used by behavioral neuroscientists.
2) Gain familiarity with neuroanatomy and other neurobiological features of the brain.
3) Independently measure different aspects of psychophysiology and prepare brain tissue for analysis.
4) Further develop research competence by using computer tools to organize, analyze, and illustrate neurobiological data.
5) Further develop critical skills in hypothesis generation and testing as well as communication skills relevant to neuroscience and psychology research.

Course Evaluations

Beyond the standard questions included in the university course evaluations, the following two questions were added:

1) What was your favorite lab activity of the semester and why?
2) What was your least favorite lab activity of the semester and why?

We then assessed the proportion of students across two sections who preferred the CURE-related activities versus the non-CURE related activities in the course.

Pre-Study Activities

To lead into the purpose of testing COMT genotype, students were provided with pre-lab lectures on the organization of the cortex and a review of genetics. Students completed a neuroanatomy lab that included identification of the frontal lobe and prefrontal cortex. They also completed

an electrophysiology lab where they arranged the different ions in the nervous system and modeled what happens during action potentials, in preparation for the EEG activities. This addressed course objectives 1-2.

Student Participants

Informed consent was obtained during class for participants in both class sections and used to teach students about the consenting process and IRB regulations. All students (n = 35) were provided information about the study and given the option to sign an informed consent for authorization to use class data in the study. The instructor repeatedly emphasized that participation as a research subject did not affect their participation as a student in the Biopsych CURE, and that being a participant was in no way linked to course grade. The use of anonymized code names for data collection in Qualtrics surveys ensured that their participation was perceived as entirely voluntary. This study was approved by and conducted in accordance with the guidelines from the Institutional Review Board at Northern Kentucky University (NKU IRB # 2452, approved January 3, 2024). Demographic data was collected from the student sample and not linked to anonymous student code names, as this data would have been self-identifying given the sample size. A total of 34 students consented, with one student being absent on the consent day. Data were collected during weeks 3-7 of the semester. All training and testing occurred within class time and did not require additional time outside of class.

Study Design

Figure 1 depicts the study flow, along with sample sizes for each genotype and sex for the study activities. COMT rs4680 genotype was determined from saliva samples collected during week 3 of the semester. During week 4 of the semester, students performed DNA isolation on their saliva samples. During week 5, students collected heart rate data while performing the n-back test. In week 6, students collected heart rate, respiration, and vagal tone data while performing the Stroop test. In week 7, students completed the BAS/BIS questionnaire and then collected EEG data while viewing a slide show of human faces depicting

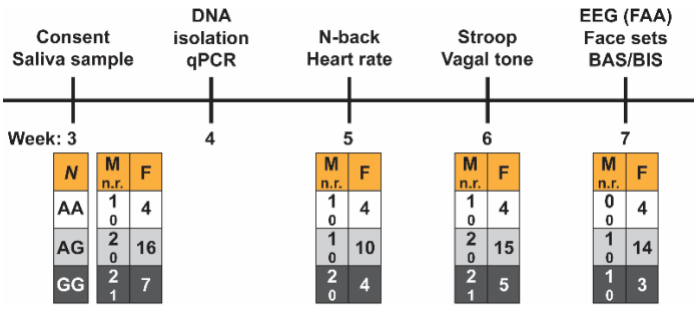

Figure 1. Study design, timeline, and sample size by genotype. The study was conducted during week 3-7 of the semester, with tables depicting sample sizes of each COMT rs4680 genotype (N) for each study activity. Due to the low sample size of male participants for each genotype, data analysis focused on female participants only. (M = male, F = female, n.r. = sex not reported)

different emotions. For data collection during weeks 5-7, students were paired with a lab partner. One partner would complete the testing while their partner would collect the data and administer any tests. Upon completion, the partners would switch roles. Students were instructed thoroughly on the procedures, given time to practice with the equipment, and data quality was monitored by the instructor.

DNA Extraction

To prepare for COMT genotype testing outside of class, students read and completed questions about an article by (Tartar et al., 2020). Saliva samples were then collected on week 3 of the semester. One student who consented to the study was absent during DNA collection, so a total of n = 33 anonymized student DNA samples were obtained. Students were instructed to abstain from food or drink for 30 minutes prior to saliva collection. Samples were incubated overnight at 50°C, and isolated during week 4 using DNA collection kits from DNA Genotek (Ottawa, Ontario, Canada). Isolation was performed by the students according to the manufacturer's protocols. To prepare for DNA isolation, one 75-minute class period was spent going over how to use pipettes for different volumes, how to centrifuge samples, and how to use a vortex. A second class period was utilized to practice creating pellets and collecting supernatant without disturbing the pellets. This included a "pellet under the hinge exercise" in which students centrifuged samples with the hinge facing outward so that the pellet was on the side closest to the hinge and they could pipette the supernatant from the opposite side. This exercise was helpful prior to DNA extraction, since DNA samples may not always produce visible pellets but students knew where the pellets were expected to be located. The DNA isolation protocol was then converted to an infographic by the instructor, with pictures making it easier to complete during class time. For their assignment, students then created a "DNA isolation hot take" where they made a brief funny video describing the protocol. The DNA isolation CURE activities addressed course objectives 1 and 3.

RNA Quantification and Real-Time PCR (qPCR)

Students then learned how to assess RNA concentration and purity using a NanoDrop spectrophotometer. Students took turns pipetting anonymized DNA samples onto the spectrophotometer, reporting 260/280 values, reporting concentrations, and diluting DNA for qPCR. This was conducted in small groups. While groups took turns with the spectrophotometer, the rest of the class practiced using the ThermoFisher assay search tool to identify assays for other common SNPs, as well as learning which allele was tagged with FAM and which allele was tagged with VIC. They used SNPedia to search for other SNPs that may interest them. The genotyping assay for rs4680 was obtained from ThermoFisher and executed according to manufacturer's protocols. Quantitative Polymerase Chain Reaction was used for detection of the rs4680 genotype using a 7500 Applied Biosystems instrument. The genotype calls for AA, AG, and GG were made based on relative VIC and FAM dye detection. The following DNA samples were obtained from the NIGMS Human Genetic Cell Repository at the Coriell

Institute for Medical Research: [HG00114, HG00115, HG00116, HG00117, HG00119]. These samples served as positive controls for each genotype and were tested alongside the Biopsych CURE study samples. Students were blinded to the results of their genotype until after the study was completed. The qPCR activity addressed course objective 1.

N-back and Heart Rate Testing

During week 5 of the semester, students had a pre-lab lecture that covered autonomic control of heart rate and then used PT-104 pulse transducers and an IXTA unit (iWorx Systems, Inc.) to record their max, min, and mean heart rate in beats per minute via Labscribe v4 or 24 software. This utilized the Vigilance-Reaction Time settings in the Human Psychophysiology module of the software. The instructor created a PowerPoint lab manual depicting the hardware and software setup and lab partners progressed through the lab together while the instructor assisted teams individually throughout the class time. To prepare for this lab outside of class, students read an article and answered questions on heart rate regulation and cognitive control (Ito et al., 2011). Students began the lab by recording their baseline heart rate for 1 minute. They then obtained heart rate values while performing the 1-back, 2-back, and 3-back tests in a randomly selected order. They were briefed on how to administer and complete the n-back, along with given an answer key document. After each trial of the n-back test, students completed a 30 second baseline measurement to reset for the next trial but these baseline values were not included in the analysis. Each n-back test consisted of a list of 10 letters read aloud by their lab partner to which the student being tested had to answer yes or no, indicating whether they recalled the same letter being read 1, 2, or 3 letters previously. Total errors were recorded. Any reported heart rate values > 200 beats per minute were discarded as inaccurate. This lab was completed in two 75-minute class periods and addressed course objectives 3 and 4.

Vagal Tone and Stroop Test

During week 6 of the semester, students had a pre-lab lecture on vagal tone and heart rate variability and a PowerPoint lab manual depicted the steps needed to complete the lab in class. Students prepared for the lab prior to class by reading and answering questions on an article investigating rs4680 genotype and how it may relate to heart rate variability in the context of major depression (Woody et al., 2014). During lab, students used PT-104 pulse transducers, RM-204 respiration belts, and an IXTA unit (iWorx Systems, Inc.) to record their max, min, and max-min heart rate in beats per minute across individual breaths (vagal tone) via Labscribe v4 or 24 software. This utilized the Personality-Vagal Tone settings in the Human Psychophysiology module of the software. Participants first obtained vagal tone under baseline conditions across 5 breaths. Heart rate and vagal tone were then measured during congruent and incongruent trials of the Stroop color-word test, presented in randomized order across groups. Students accessed the Stroop test at the following website: https://psych.hanover.edu/javatest/cle/Cognition_js/exp/stroop.html. Default settings were used, except the number of trials per condition was changed from 20 to 25 to assess more breath cycles. Participants did not know which trial came first (congruent or incongruent). For each trial (baseline, congruent, and incongruent) maximum heart rate, minimum heart rate, and vagal tone were extracted. Speed and accuracy of the congruent and incongruent trials were also recorded. Any reported heart rate values > 200 beats per minute were discarded as inaccurate. This lab was completed in two 75-minute class periods and addressed course objectives 3 and 4.

Behavioral Activation Scale and Behavioral Inhibition Scale

During week 7 of the semester, students had a pre-lab lecture on EEG waves, frontal alpha asymmetry, and how this could relate to personality. Students completed the Behavioral Activation and Behavioral Inhibition Scales (BAS/BIS) via Qualtrics. There were 20 questions scored on a four-point Likert scale (very false for me = 1, somewhat false for me = 2, somewhat true for me = 3, very true for me = 4) (Carver and White, 1994). Scored categories included averaged values for the following: BIS or punishment sensitivity, BAS reward responsiveness, BAS drive, BAS fun-seeking, and average BAS.

EEG Frontal Alpha Asymmetry

Immediately after completing the BAS/BIS questionnaire, students used C-ISO-SL7 EEG snap leads with an iWire headband, iWire-B3G interface, and IXTA unit (iWorx Systems, Inc.) to record alpha and beta wave activity via Labscribe v4 or 24 software. This utilized the EEG-Cortical Arousal settings in the Human Psychophysiology module of the software. A PowerPoint lab manual contained pictures of all hardware and software settings to facilitate learning to use the equipment. Four leads were placed across the front of the forehead to measure frontal cortical activity, with two leads measuring activity in each hemisphere. An additional ground electrode was centered over the middle of the forehead. For all EEG measurements, participants recorded left and right alpha and beta frequencies (Hz) and amplitude (mV). Participants first obtained a baseline measurement for 1 minute. They then sat quietly and viewed a 2 min automated PowerPoint presentation that presented 36 s of emotional faces (6 different faces depicting the same emotion, each displaying for 6 s). After each 36 s block of 6 face presentations with the same emotion, there was a 4 sec intertrial interval. Participants viewed a total of three 36 s emotional face presentations, depicting neutral, angry, and happy faces in a randomized order (Tottenham et al., 2009; Conley et al., 2018). The instructor provided PowerPoint files of the emotional face set presentations, which were automatically timed when viewed in presentation mode. EEG measurements were collected for each of the emotional face sets, during the last 25 seconds of the 36 s presentation. Frontal alpha asymmetry (FAA) was calculated by the instructor as the natural log of the right hemisphere alpha power (mV^2) minus the natural log of the left hemisphere alpha power (mV^2) (Coan and Allen, 2004; Sun et al., 2017). For their assignment, students created an

"EEG meme" where they included how FAA is defined and how that was proposed to relate to personality. This lab was completed in two 75-minute class periods and addressed course objectives 3 and 4.

Statistical Analysis

Utilizing the two questions on the course evaluations, the proportion of students preferring CURE versus non-CURE activities as their favorite or least favorite among the course was analyzed via one-sided χ^2 test, since it was explicitly predicted that a greater proportion of students would rank CURE activities as their favorite and fewer would rank CURE activities as their least favorite. Students were taught to conduct data analysis in class using Jamovi 2.3.28 (The Jamovi Project, 2024). The instructor collected anonymized student data using a Qualtrics survey for each lab activity. Students were presented with the final cleaned and organized data with which they were taught to conduct statistics. The instructor created a summarized guide for all statistical testing and settings. The statistical analyses addressed course objective 4. The instructor further analyzed and graphed data using GraphPad Prism 10. Two-way repeated measures ANOVAs (or mixed-effects models for data sets with missing values) were used to assess the effect of COMT rs4680 genotype on heart rate, vagal tone, and FAA across trials of the various tests. Additional two-way repeated measures ANOVAs were used to assess the effects of COMT rs4680 genotype on errors in the n-back, speed in the Stroop task, and accuracy in the Stroop task across various trials of each test. The Greenhouse-Geisser correction was applied for all ANOVAs. One-way ANOVAs were used to assess BAS/BIS scores based on genotype. Tukey's post hoc testing was used to evaluate overall genotype effects between individual genotypes or overall trial effects between individual trials when main effects of genotype or trial were present, respectively. Individual group differences during specific trials were not assessed in the absence of a significant genotype by trial interaction. Data are depicted as mean ± standard error of the mean.

Student Research Reports

Students learned to use Jamovi to graph their data, and together with their lab partner, wrote an APA-style manuscript containing the results, which addressed course objective 5. Students were provided with the entire data set from all CURE activity labs and permitted to select three findings to present in their research report. They could utilize hypotheses presented in pre-lab lectures or generate their own based on the data provided.

RESULTS

Course Evaluations

In the first and second sections of the Biopsychology Laboratory course, 50% and 83% of students reported that at least one activity within the Biopsych CURE was their favorite activity of the semester, based on 76% and 54% response rates. The remaining students listed other active learning laboratory exercises that were not part of the 5-week Biopsych CURE as their favorite. Students described the genotyping as exciting, enjoyed using EEG and heart rate monitoring, liked performing activities with their lab partner, and appreciated the conceptual organization of the Biopsych CURE as an authentic research study. Across the two sections, only 44% and 40% of students reported that an activity within the Biopsych CURE was their least favorite activity of the semester, with 76% and 50% response rates. Statistically, the proportion of students ranking a CURE activity as a favorite activity was higher and the proportion ranking a CURE activity as a least favorite activity was lower in comparison to non-CURE activities in the course, $\chi^2_{(1)} = 2.8$, $p = 0.047$ (Figure 2). Every comment that listed an activity in the Biopsych CURE as their least favorite indicated that this was due to working with saliva as a bodily fluid and the occasional inconsistent readings obtained with the iWorx equipment.

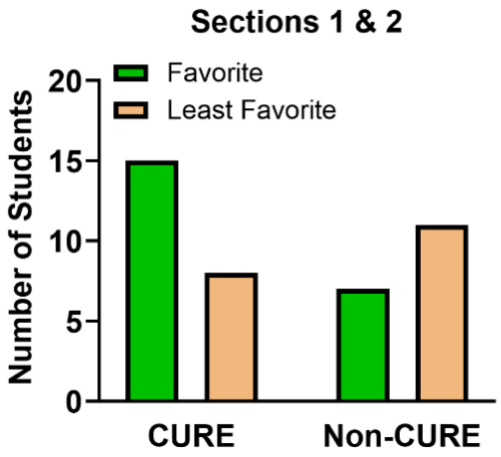

Figure 2. Students were more likely to rank a CURE activity as a favorite activity and a non-CURE activity as a least favorite activity.

Participant Demographics

Thirty-one students completed the demographics survey (age: M = 22.5 years, SD = 5.9 years). The study sample consisted of 4 male (age: M = 26.5 years, SD = 8.3 years), 25 female (age: M = 21.9 years, SD = 5.7 years), 1 nonbinary, and 1 participant who preferred not to say. There were 29 students who were White or European American (n = 23 female, n = 4 males), 1 who was Asian American (female), and 1 who was Asian (female). There were 22 students who completed the N-back testing (n = 4 male, n = 18 female), 30 students who completed the Stroop and vagal tone testing (n = 5 male, n = 24 female, n = 1 that did not report sex), and 24 students who completed the BAS/BIS and EEG testing (n = 2 male, n = 22 female). Due to the low number of non-female participants, our analyses focused on the female participants.

COMT rs4680 Genotype

Study-wide, the genotype frequencies were AA (n = 5; 15.15%), AG (n = 18; 54.54%), and GG (n = 10; 30.3%). This did not differ significantly from the expected frequencies based on the Hardy-Weinberg principle, $\chi^2_{(2, 33)} = 0.256$, $p = 0.88$. The genotype counts for each of the study activities based on sex are presented in Figure 1.

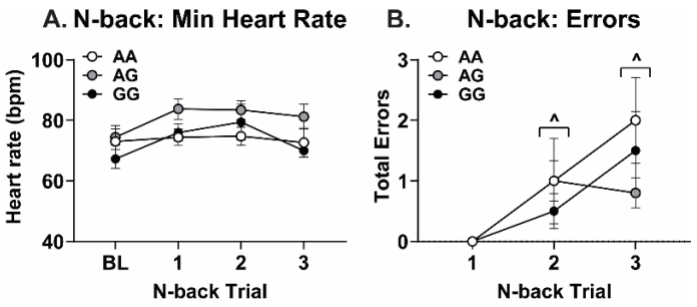

Figure 3. Heart rate and n-back testing. (A) There were no COMT rs4680 genotype differences in minimum heart rate for the baseline assessment or across the 1, 2, and 3-back trials of the n-back. (B) Total errors were increased in the 2 and 3-back trials compared to the 1-back trial, but this did not depend on genotype (^ $p < 0.05$ vs. 1-back after trial post hoc comparisons based on trial main effect). (bpm = beats per minute)

N-back and Heart Rate Testing

Minimum, maximum, or mean heart rate while performing the n-back test did not reveal any main effects of COMT rs4680 genotype, main effects of n-back trial, or interactions between genotype and trial. See Figure 3A for minimum heart rate results. There was a main effect of trial on total errors in the n-back, $F_{(1.5, 31.8)} = 10.47$, $p = 0.0009$ (Figure 3B). Post-hoc testing revealed that students had more errors in the 2-back and 3-back tests compared to the 1-back test ($p < 0.05$) (Figure 3B). However, there was no main effect of genotype and no trial x genotype interaction.

Vagal Tone and Stroop Test

Vagal tone differed significantly based on COMT rs4680 genotype across trials, $F_{(2, 60)} = 9.37$, $p = 0.0003$ (Figure 4A). Participants with the GG genotype had higher vagal tone compared to those with the AA and AG genotypes ($p < 0.05$) (Figure 4A). Minimum heart rate also differed significantly based on genotype across Stroop test trials, $F_{(2, 21)} = 4.2$, $p = 0.029$ (Figure 4B). However, post-hoc testing did not reveal any significant differences in minimum heart rate between specific genotypes. There were no main effects or interactions for maximum heart rate or mean heart rate. Finally, there was a significant trial effect for average trial completion time and average accuracy, with participants having longer completion times and lower accuracy for the incongruent trials compared to the congruent trials, [$F_{(1, 20)} = 4.6$, $p = 0.043$; $F_{(1, 21)} = 5.225$, $p = 0.032$] (Figure 4C-D). Time and accuracy did not vary based on genotype.

EEG Frontal Alpha Asymmetry (FAA)

EEG data from two participants were removed, with one participant failing to complete the emotional face set presentation and another participant submitting alpha and beta amplitudes that were not within an acceptable range. FAA while viewing different emotional face sets differed significantly based on COMT rs4680 genotype, $F_{(2, 17)} = 3.94$, $p = 0.039$ (Figure 5A). Post hoc testing showed that individuals with the GG genotype had higher FAA compared to those with the AA genotype ($p < 0.05$). This did not depend on emotional face trial, as there was no effect of trial and no trial x genotype interaction. Alpha and beta amplitude in the left and right cortex did not differ based on genotype or trial, and there were no interactions between these variables.

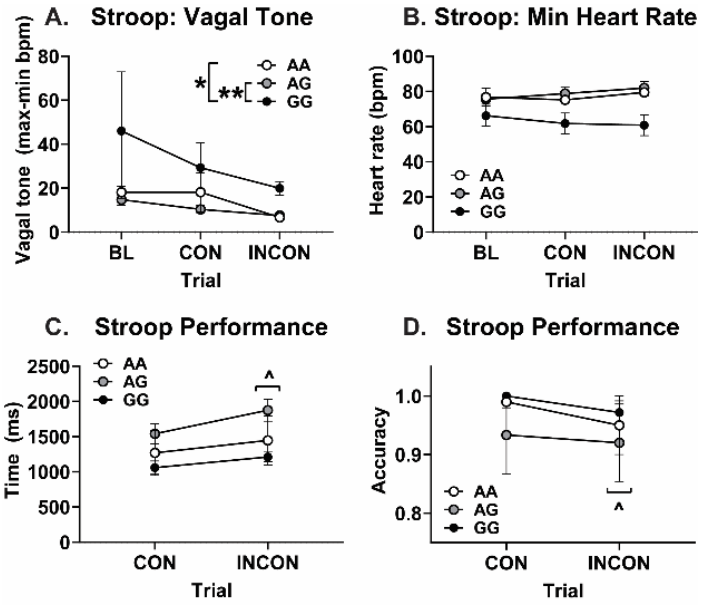

Figure 4. Vagal tone and Stroop testing. (A) The GG COMT rs4680 genotype had higher vagal tone than the AG and AA genotype across the baseline measurement, congruent, and incongruent Stroop test but there was no effect of trial (* $p < 0.05$ vs. AA and ** $p < 0.05$ vs. AG after genotype post hoc comparisons based on genotype main effect). (B) There was a main effect of genotype on minimum heart rate across the Stroop test trials, but no comparisons reached significance. (C) Average trial completion was higher and (D) trial accuracy was lower during the incongruent Stroop trials compared to the congruent Stroop trials, but there was no effect of genotype (^ $p < 0.05$ vs. congruent). (BL = baseline, CON = congruent, INCON = incongruent, bpm = beats per minute, min = minimum)

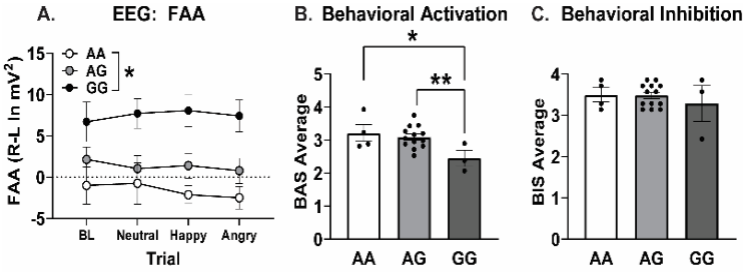

Figure 5. (A) The GG COMT rs4680 genotype had higher frontal alpha asymmetry values (FAA) than the AA genotype across the baseline measurement and different emotional face set presentations but there was no effect of trial (* $p < 0.05$ vs. AA after genotype post hoc comparisons based on genotype main effect). (B) The GG genotype had lower behavioral activation scores (BAS) compared to the AA and AG genotype (* $p < 0.05$ vs. AA; ** $p < 0.05$ vs. AG). (C) Behavioral inhibition scores (BIS) did not vary based on genotype. (BL = baseline, R = right, L = left)

BAS/BIS Scores

Average BAS scores differed significantly based on COMT rs4680 genotype, $F_{(2, 17)} = 4.12$, $p = 0.035$ (Figure 5B). Individuals with the GG genotype had lower BAS scores compared to both AA and AG individuals ($p < 0.05$) (Figure 5B). There were no differences in the BAS subscores: drive [$F_{(2, 17)} = 0.95$, $p = 0.41$], reward [$F_{(2, 17)} = 3.4$, $p = 0.056$], or fun [$F_{(2, 17)} = 3.58$, $p = 0.051$]. Average BIS scores did not vary based on COMT genotype (Figure 5C).

DISCUSSION

Through this Biopsych CURE, 35 students gained practical research experience with conducting a multifaceted neuroscientific study on human participants. They learned about DNA collection and genotyping via quantitative PCR by actively completing many of the steps in these procedures themselves. Implications of the genotype studied focused on variability in dopamine neurotransmission in the prefrontal cortex. Biopsych CURE students learned how to operate equipment for collecting heart rate and EEG data, and how to relate these concepts to brain function, with a specific focus on the prefrontal cortex. Students engaged in prefrontal cortical behavioral tasks both as participants and as investigators, providing a well-rounded experience for implementing these tests in a research setting. Students were assigned research articles to read that solidified their understanding of the CURE activities. Finally, students conducted statistical analysis, graphed results from the study, and completed a research report. Overall, students saw how all of these activities integrated together to test a research hypothesis.

The CURE implementation addressed all five course objectives and students ranked CURE activities as being more preferred out of the other activities conducted in the course. Students genuinely appreciated the hands-on approach and were invested in the study outcomes. Pre-lab lectures were minimal but targeted, with material carefully selected to align with CURE activities. Providing the students with an organized research project and a lab partner enabled them to work at their own pace during class time, with the instructor able to assist students on a more individual level when they did need help. Students commented they enjoyed this approach, as it helped them troubleshoot independently and gain confidence in formulating and asking questions.

This Biopsych CURE demonstrated that among female undergraduate students, the COMT rs4680 GG genotype had higher vagal tone and higher frontal alpha asymmetry (FAA) that was not dependent on the stimulus type evaluated. Evaluated stimuli included cognitive conflict via the Stroop test and emotional face set presentations, respectively. Participants with the GG genotype also had lower self-reported behavioral activation scores (BAS) but did not differ in their behavioral inhibition scores (BIS). COMT genotype was not associated with changes in heart rate either at baseline or under cognitive load, measured via the n-back test. Finally, we did not observe genotype differences in executive function performance on the Stroop test or n-back test, although this could relate more to the relative ease of these tests under the testing circumstances. We observed higher FAA in GG participants, which was contrary to our hypothesis, and this did not relate to an increase in self-reported behavioral approach/activation (BAS). Rather, we observed higher behavioral approach/activation scores in the A carriers. This aligns with enhanced reward responsivity, reward-seeking behavior, and reward-based learning observed in the A genotype (Lancaster et al., 2012; Corral-Frías et al., 2016). BAS has been positively correlated with FAA but dopamine receptor antagonism has been capable of reversing this relationship (Wacker et al., 2013). This state of lower dopamine tone is recapitulated with the G genotype having reduced prefrontal dopamine levels and the opposite expected relationship between FAA and BAS: higher FAA values yet lower BAS scores in our current study.

We found that the homozygous GG genotype had higher vagal tone, independent of the different trials of the Stroop test. This suggests overall greater parasympathetic activity and higher HRV in GG individuals compared to the homozygous AA and heterozygous AG individuals (Laborde et al., 2017). This supports previous work in young adults showing that GG individuals had increased high frequency HRV power (Chang et al., 2019). It also supports a prior finding in female participants, where the A allele has been associated with lower HRV among those with major depressive disorder (Woody et al., 2014).

An interesting observation is that the GG genotype displayed lower BAS scores but higher vagal tone in our study. Low dopamine levels have been linked to BAS deficits and when combined with low vagal tone, this can increase the risk of aggression and risk-seeking (Beauchaine et al., 2007). However, it appears that the GG genotype is protected from displaying this psychophysiological pattern.

We observed no differences in minimum, maximum, or mean heart rate based on COMT rs4680 genotype. This demonstrates that HRV/vagal tone and possibly parasympathetic activity are more affected by genotype than sympathetic activity. One prior study has shown that A carriers displayed increased heart rate among those who report higher daily caffeine intake (Brathwaite et al., 2011), so this may only be observed under situations that increase catecholamine levels dramatically.

Overall, our study size was small and the results should be interpreted as a guide for implementing the Biopsych CURE and aiding student hypothesis generation rather than as a formal research study. With plans to continue implementing this CURE, we can increase student assessments to collect more detailed feedback in the evaluations. The instructor also encouraged students to formulate their own research questions from the course data, but this was challenging for them and even more challenging to integrate into their research reports. We overcame this challenge by dedicating more class time to the research report. However, we plan to make this more

guided in the future, particularly by organizing data into possible themes to choose from, so that the students can choose concepts that are more interrelated. Another future goal is to have students present their work at local conference events. Further, this CURE can be adapted to test other SNPs that may be involved in biopsychological function in the future.

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