RESEARCH ARTICLE

Cholinergic-estrogen interaction is associated with the effect of education on attenuating cognitive sex differences in a Thai healthy population

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Abstract

The development of human brain is shaped by both genetic and environmental factors. Sex differences in cognitive function have been found in humans as a result of sexual dimorphism in neural information transmission. Numerous studies have reported the positive effects of education on cognitive functions. However, little work has investigated the effect of education on attenuating cognitive sex differences and the neural mechanisms behind it based on healthy population. In this study, the Wisconsin Card Sorting Test (WCST) was employed to examine sex differences in cognitive function in 135 Thai healthy subjects, and label-free quantitative proteomic method and bioinformatic analysis were used to study sex-specific neurotransmission-related protein expression profiles. The results showed sex differences in two WCST sub-scores: percentage of Total corrects and Total errors in the primary education group (Bayes factor>100) with males performed better, while such differences eliminated in secondary and tertiary education levels. Moreover, 11 differentially expressed proteins (DEPs) between men and women (FDR<0.1) were presented in both education groups, with majority of them upregulated in females. Half of those DEPs interacted directly with nAChR3, whereas the other DEPs were indirectly connected to the cholinergic pathways through interaction with estrogen. These findings provided a preliminary indication that a cholinergic-estrogen interaction relates to, and might underpin, the effect of education on attenuating cognitive sex differences in a Thai healthy population.

Introduction

Human brain development is influenced by both genetic and environmental factors. The X and Y chromosomes define not just a person's sex, but also the morphological and functional distinctions between male and female brains. For example, the male brain tends to be bigger with a higher proportion of white matter [1], whereas the female brain generally has a larger corpus callosum [2]. According to Ingalhalikar et al. [3], the male brain is thought to be more intrahemispherically connected, whereas the female brain appears optimized for interhemispheric connectivity. There is growing acknowledgment that the human brain is sensitive to environmental circumstances throughout development, such as socioeconomic status, education, nutrition, and parental care [4]. Evidence suggests that children from higher-income families have greater volumes of grey and white matter in the inferior frontal gyrus and bigger hippocampus sizes than their lower socioeconomic status peers [5, 6]. Furthermore, education not only enhances human brain development but also attenuates cognitive deficits that occur with aging [7–9]. Schooling increases cortical grey matter volumes (GMV) in several brain areas [10], and even a few years of education has been demonstrated to contribute to brain cognitive reserve [11].

Under the joint actions of genetic and environmental factors, male and female brains may differ in terms of cognitive strategies and/or cognitive styles [12]. These include different learning style preferences [13], as well as sex differences in decision-making [14]. Previous research found that in correct trials of the Wisconsin Card Sorting Test (WCST), males had shorter response time, which is a measure of efficiency of the decision-making process [15], while females made fewer WCST errors [16]. However, another study conducted by Cinciute and colleagues [17] found no significant difference in the duration of WCST task performance regarding sex.

There has been minimal research into the effect of education on attenuating cognitive sex differences. Bloomberg et al. [18] discovered that males in UK had higher fluency scores only in the low education group (less than high school diploma). There was evidence of higher fluency scores in females in the high education group (high school diploma and higher), especially in those born later. This finding implied that schooling, as well as secular changes in education level across birth cohorts, had a role in determining cognitive performance in females. However, it is unknown whether this result extends to other ethnicities or cognitive domains.

Cognitive performance is the reflection of information transmitted along with neural circuits of brain areas [19, 20], and learning and obtained experiences have been documented to alter the morphology of synapses as well as promote their plasticity [21]. The study of how education affects neurotransmission in male and female brains is still in its infancy and has to be expanded. Therefore, the purpose of this study is to investigate the effects of education on cognitive sex differences in a Thai healthy population, as well as to investigate the neural mechanisms behind such effects.

Materials and methods

Study subjects

As described in a previous study from our research team [22], one hundred and thirty-five healthy volunteers between the ages of 22 and 70 were recruited. Subjects were divided into two categories based on their education level: those who had acquired no more than primary education, and those who had received secondary education, for some, tertiary education. Subjects with abnormal mental health evaluated by the Thai Mental Health Indicator (THMI-55)

were excluded from the current study. The Mini-Mental State Examination (MMSE) was also used to screen out participants with dementia. To limit the likelihood of confounding by population stratification, all individuals were of Thai ethnicity [23]. Written consent forms were obtained from the participants involved. The experimental protocols were approved by the Human Ethics Committee of Naresuan University (Naresuan University Institutional Review Board), COA No. 0262/2022.

Cognitive assessment

The Wisconsin Card Sorting Test (WCST) has long been used in clinical and research settings to assess the frontal cortex (FC) function [24]. The recent neuroimaging study revealed that, in addition to FC, a widespread network of prefrontal, frontal, temporal, frontal-temporal, and parieto-occipital regions are activated during WCST [25], and multiple domains of cognitive function including but not limited to attention, cognitive set-shifting, planning or organizing, problem-solving and decision-making are activated during the process of WCST [26].

In this study, all subjects were tested using a computer-based WCST (Inquisit 3.0.6.0), and the raw score was analyzed to reflect different domains of cognitive function, as shown below [27]:

- The percentage of total corrects (%Corrects): the entire number of correct response cards multiplied by 100 and divided by the total number of cards, reflecting initial conceptualization and attention.

- The percentage of total errors (%Errors): the total number of incorrect response cards multiplied by 100 and divided by the entire number of cards, reflecting nonspecific cognitive impairment.

- The number of categories completed (Category completed): determined by applying the score range from 1 to 6, reflecting cognitive set-shifting.

- The perseverative errors (PE): the score was used to assess the inability to correct a response due to ignorance of relevant stimuli, reflecting cognitive inflexibility.

- Trails to complete the first category (1st Category): the score ranges from 0 to 128, which is the number required to complete the initial category of the task, reflecting initial conceptualization.

Blood sample collection

A 3ml of cubital vein blood was collected from each enrolled subject immediately after they completed the WCST test. The blood was centrifuged at 3,000 rpm for 5 minutes. The serum was then transferred into a 1.5ml microcentrifuge tube and stored at -80˚C in a refrigerator for future use, all samples were coded to ensure anonymity.

Label-free quantitative proteomics analysis

The label-free quantitative proteomics analysis was used to compare serum protein expression profiles between males and females. The analytic processes were performed by the Functional Proteomics Technology Laboratory, National Centre for Genetic Engineering and Biotechnology, Pathum Thani, Thailand, which include protein digestion, Liquid Chromatography with tandem mass spectrometry (LC-MS/MS) analysis, protein identification, and protein quantitation.

In brief, the protein concentration of all serum samples was determined using the Lowry assay with BSA as a standard protein [28]. Five micrograms of protein samples were digested with sequencing grade porcine trypsin (1:20 ratio) for 16 hours at 37 ˚C. The tryptic peptides were dried in a speed vacuum concentrator and resuspended in 0.1% formic before further analysis.

The prepared tryptic peptide sample of each subject was injected individually into an Ultimate3000 Nano/Capillary LC System (Thermo Scientific, UK) coupled to a Hybrid quadrupole Q-Tof impact II™ (Bruker Daltonics) equipped with a Nano-captive spray ion source. Mass spectra (MS) and MS/MS spectra were obtained in the positive-ion mode at 2 Hz over the range (m/z) 150–2200. To minimize the effect of experimental variation, three independent MS/MS runs were performed for each sample.

MaxQuant 1.6.6.0 was used to quantify and identify the proteins in each sample with the Andromeda search engine to correlate MS/MS spectra to the Uniprot *Homo sapiens* database [29]. The proteins were identified using a 10% protein false discovery rate (FDR), carbamido-methylation of cysteine as fixed modification, and the oxidation of methionine and acetylation of the protein N-terminus as variable modifications. Only proteins with at least two peptides, and at least one unique peptide, were considered as being identified and used for further data processing.

Bioinformatic analysis

Before any analysis, data cleansing and preprocessing were performed by using Perseus ver. 1.6.15.0 [30]. A list containing all proteins identified previously was submitted to jvenn (web application) to explore those proteins shared by males and females in both primary and secondary and tertiary education groups [31]. Differentially expressed proteins (DEPs) between males and females in each education group were detected independently using the Linear Model for Microarray Data (LIMMA) approach within R-programming ver. 4.1.2 [32], with the FDR set at 10%. Multi-Experiment Viewer (MeV, ver.4.9.0) software was employed to illustrate the expressions of shared DEPs in men and women from both education groups [33]. The protein-protein interaction (PPI) network, as well as the relationship between DEPs and cognitive function, was investigated using Pathway Studio ver. 12.5 [34, 35].

Statistical analysis

Using R-programming ver. 4.1.2, the multi-factorial analysis of variance (ANOVA) was performed to study the main effects of sex, age and education, as well as their interactions, on WCST scores. A General Linear Model (GLM) approach paired with Bayesian statistics was applied to analyze differences in WCST scores between men and women at each education level [36], as well as comparisons of males and females across the two education levels, with age as a covariate. Bayes factor (BF) was offered to illustrate the likelihood of supporting the alternative hypothesis [37, 38], and the sensitivity analysis was also employed to test the robustness of the results [39]. BF≥10 or P≤0.05 was considered significant in this study.

Results

Demographic characteristics of the study population

The Subjects were 70 males and 65 females, with a mean age of 57.75±10.35 years (range, 22–70 years). Table 1 describes their demographic data, including sex, age, and educational level. The study found that there were significant age differences between males and females in the secondary and tertiary education groups, but not in the primary education group. However,

Table 1. Demographic data of subjects.

	Male	Female	BF	95% CI
Primary education				
No. of subjects	49 (70%)	42 (64.6%)		
Age	61.4±6.02	62.5±6.81	1.56	[-3.78, 1.66]
Secondary and Tertiary education				
No. of subjects	21 (30%)	23 (35.4%)		
Age	51.1±13.2	47.3±9.75	5.73	[-3.18, 10.88]

Data was presented as mean±SD by General Linear Model.

BF = Bayes Factor, 95% CI = 95% Credible interval of difference male against female.

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age difference between the two education groups was discovered with decisive evidence (BF>100, 95%CI = [9.65, 16.0]).

The main effects of sex, age, and education, as well as their interactions, on WCST scores

The results of multi-factorial ANOVA revealed that sex, age and education level had significant main effects on two of the five WCST-sub scores %Corrects and %Errors (see Table 2). Aside from that, there were significant interaction effects of sex by age (P<0.01) on scores %Corrects, %Errors and Category Completed, as well as significant age by education interaction effect on 1st Category (P<0.05).

Effects of education on sex differences in cognitive performance

The Jeffreys-Zellner-Siow (JZS) priors [40] is the default prior used in the general linear model, with a default r scale of 0.353. The data suggested that education have potential compensatory effects on three out of the five WCST sub-scores: %Corrects, %Errors, and 1st Category. In the primary education group, male scored better in %Corrects (BF>100, 95%CI = [2.74, 12.25], Cohen's d = 0.68) and had lower %Errors (BF>100, 95%CI = [-12.2, -2.71], Cohen's d = 0.67) with close to large effect size [41]. While sex differences in those two scores reversed in the higher education group, however, there was only weak evidence to support the alternative hypotheses (both BF<2) (see Fig 1 and full data set in S1 Table). Regarding the score 1st Category, females were found to have fewer trials to complete the first category in the WCST test compared to males in both education groups, but there was weak evidence to reject the null hypothesis in the lower education group (BF = 1.02). Such sex difference was decisive in the second and tertiary education group (BF>100, 95%CI = [-6.65, 15.8]), although the effect size was small (Cohen's d = 0.25). The results remain robust after sensitivity analyses.

Table 2. Main effects of sex, age and education on WCST sub-scores.

	%Corrects	%Errors	1st Category	Category Completed	PE
Sex	P = 0.05*	P = 0.05*	P = 0.32	P = 0.73	P = 0.08
Age	P = 0.01*	P = 0.01*	P = 0.50	P = 0.26	P = 0.68
Education	P = 0.03*	P = 0.02*	P = 0.26	P = 0.58	P = 0.28

*P≤0.05 by multi-factorial ANOVA

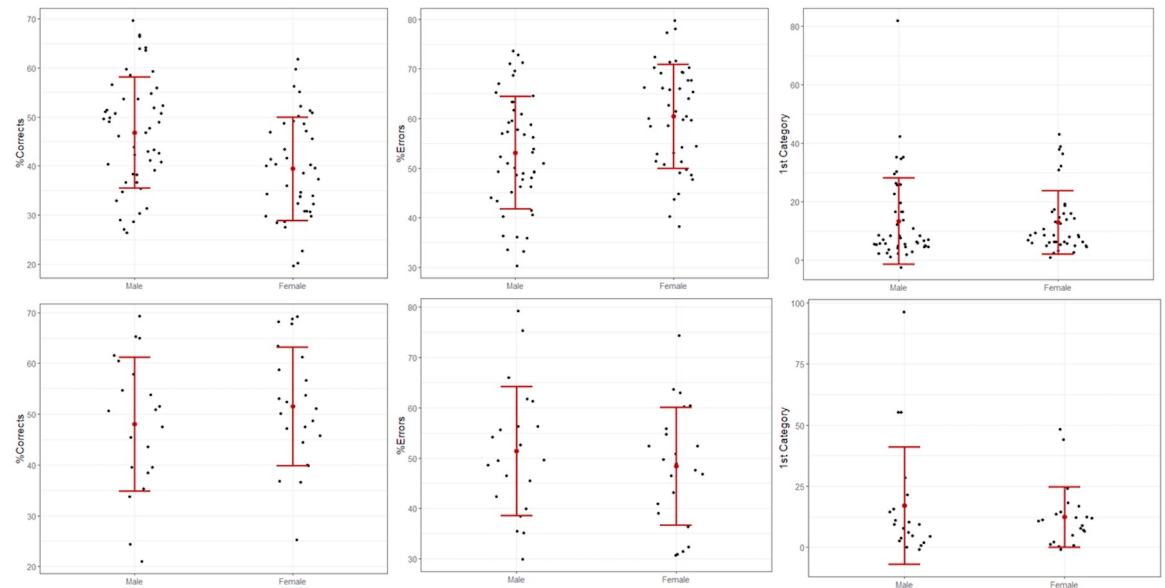https://doi.org/10.1371/journal.pone.0278080.t002

Fig 1. Effects of education on sex differences in WCST sub-score %Corrects, %Errors, and 1st Category. The upper graphs show sex differences in those scores in the primary education group, the lower graphs depict the sex differences in the same scores in the secondary and tertiary education groups.

Furthermore, there was insufficient evidence to accept the sex differences in score PE and Category Completed in both education groups (all BF<6).

In addition, females with secondary and tertiary education performed significantly better in scores %Corrects and %Errors (BF>100) than those in the lower education group, and moderate evidence for such difference in the score 1st Category (BF = 4.55). While limited evidence indicated differences in scores of %Corrects (BF = 4.79), %Errors (BF = 4.43), and 1st Category (BF = 3.48) in males across the two education levels.

Identification and relative quantification of differentially expressed proteins between men and women

After applying the criteria outlined in section 2.4, a total of 886 proteins were identified using a label-free quantitative proteomics approach, with 808 of them shared by both sexes (see Fig 2). Of those 808 proteins, we found that 11 differentially expressed proteins (DEPs) between men and women (FDR<0.1) were present in both education groups (see Fig 3 and full data set in S2 Table). Except for Hematopoietic progenitor cell antigen CD34 (CD34), all of the 11 DEPs were upregulated in females.

Protein-protein interactions of DEPs and their relationship with cognitive function

As shown in Fig 4, out of the 11 DEPs, LRP4, TTR, TRIM38, and PP2B interacted directly with nAChR3, a subunit of nicotinic acetylcholine receptor that positively regulates cognition. Of the others, CD34, SERPING1, HSD17B1, KNG1, and TTR are indirectly connected to the cholinergic pathways through interaction with estrogen. This suggested that the cholinergic-estrogen interaction may have an influence on cognitive processes. STRCP1 and RAB4A, on the other hand, showed no interaction with other DEPs or association with cognitive function.
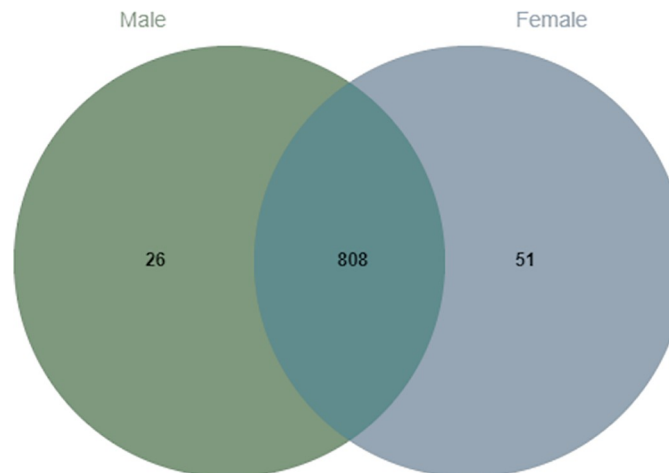
Fig 2. Venn diagram showing 808 proteins shared by both males and females.

Discussion

In this study, we observed sex differences in WCST sub-scores %Corrects and %Errors in the primary education group, with males outperforming females, which is consistent with earlier research [42], but these differences were not supported by sufficient evidence in the higher education group. The WCST is a test of cognitive flexibility, that is the ability to adjust behavioral response mode in the face of changing conditions [25, 43]. Its sub-scores %Corrects and %Errors show specific domains of cognitive function, which correspond to the activity of certain brain regions. For example, %Corrects reflects conceptualization and attention, and a previous study showed that multiple areas of the prefrontal cortex (PFC) may participate in information processing during attentional shift in the WCST [44, 45]. While %Errors reflecting non-specific cognitive impairment and frontal and temporal lobes lesions have been linked to a large number of WCST total errors [46, 47].
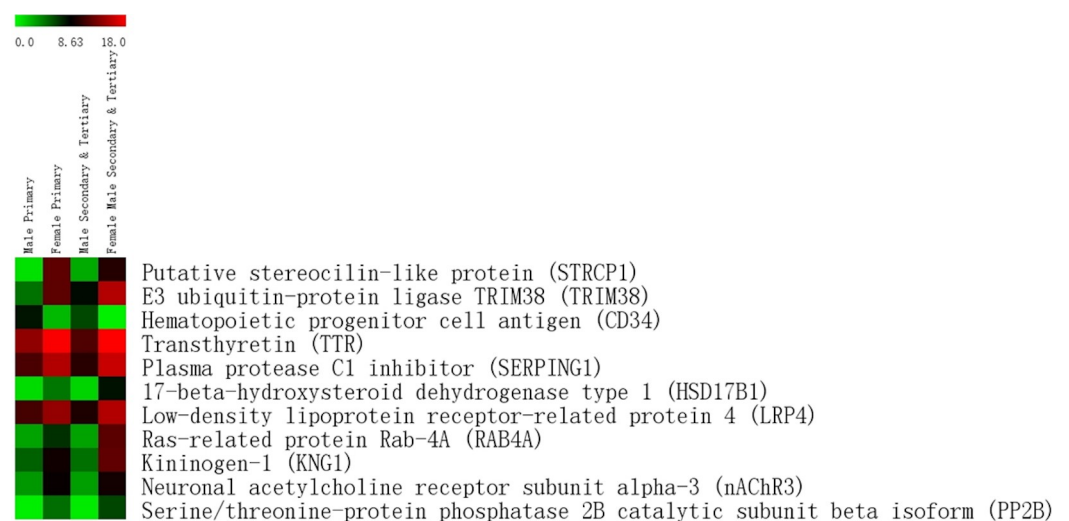

Fig 3. Expression heatmap of 11 proteins exhibited in both education groups.
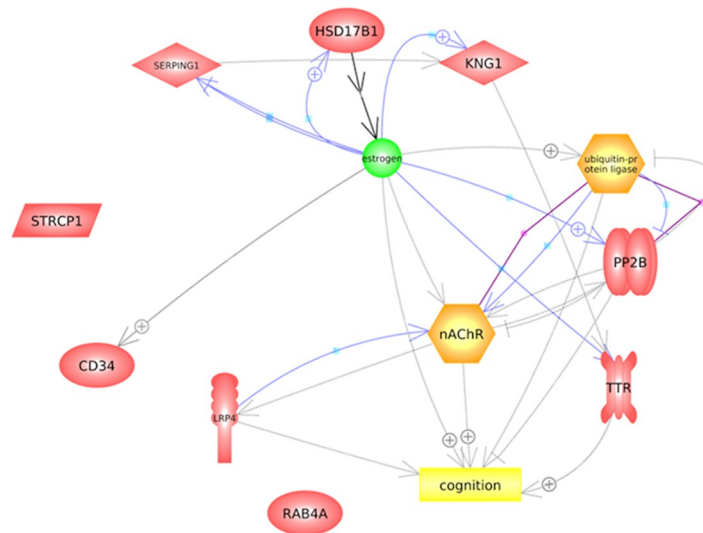
Fig 4. Protein-protein interaction network produced by Pathway Studio. The purple line indicates binding, the blue line indicates expression, the black line indicates chemical reactor, the solid gray indicates molecular transport, while the gray dotted line indicates regulation. The plus sign indicates positive regulation.

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The WCST results in this study suggested that education may have an effect on attenuating cognitive sex differences. This is consistent with previous research demonstrating that sex differences in attention control to spatial cognition can be eliminated through short-term instruction or long-term experience in life [48–50]. Longer education led to increased cortical thickness in bilateral temporal, parietal, medial-frontal, sensory, and motor cortices in the adult brains [51]. Even after six weeks of multi-component cognitive control training, adolescents showed increased GMV in the right inferior frontal cortex (rIFC) [52]. In contrast, Rzezak et al. [53] discovered subtle or no differences in both whole-brain and regional GMV between participants with high and low education levels. Furthermore, a prior study found that females benefit more from certain training than males, and follow-up testing suggested that the gain was persistent [50]. This suggests a possible explanation for the effect of education on attenuating cognitive sex differences in WCST in terms of brain morphology: Formal education, which contains a curriculum for knowledge acquisition as well as physical and awareness development [54–56], promotes brain development in both sexes, particularly in the frontal cortex [57], which involved in attention and set-shifting in WCST [44, 58], and such effects may be accentuated in women, attenuating the cognitive sex differences in these two WCST scores. This idea was partially supported by earlier findings that education as well as closing the gap between males and females in educational opportunities, promote cognitive performance in females [18], and the skills required for efficient completion of the WCST may be closely associated with those taught and required in formal education [16]. In contrast, females did worse in the primary education group for both %Corrects and %Errors, with fewer correct responses and more errors. The total errors of WCST reflect non-specific cognitive impairments, and another research we conducted suggested that females might be more susceptible to the excitotoxicity induced by WCST [59]. This, combined with the loss of estrogen's neuroprotection effects after menopause [60–62], led to loss of brain environment homeostasis and impaired cognitive function in elderly female subjects [59].

On the other hand, healthy participants were thought to be more or less homogeneous on WCST performance because no sex difference was observed [63, 64]. Previous clinical research

has demonstrated that GMV is connected to WCST performance [65, 66]. Despite a male advantage in GMV, females have higher gray matter density than males throughout the brain, which may compensate for the lower GMV in females [67], leading to no significant sex differences in general intelligence [68].

Additionally, females outperformed males in score 1st Category in this study, but only in the higher education group. This is consistent with previous findings [16]. This score reflects initial conceptualization abilities, and numerous research findings support the idea that lateral PFC plays a central role in rule-based reasoning [69, 70], which further suggested that female brains, especially the PFC region, may be more sensitive to the boost effects of education. The other two WCST scores, Category Completed and PE, did not demonstrate significant sex differences in the current research, which contradicts earlier studies [16, 71]. Perhaps the relative low number of subjects following stratification by education in each education group prevents us from detecting sex differences in these two scores [16]. Interestingly, Cinciute et al. [17] identified significant sex-related neuronal activity in the PFC in healthy participants, but behavioral results of the WCST did not show sex bias. This contradiction arises because some functional brain maps of the WCST may be sensitive to neurophysiology heterogeneity which is not apparent or necessarily related to the behavior [17]. Although the standardization, validity and reliability of WCST as a stand-alone cognitive assessment in healthy adults of various ages and education levels have been established [72], a battery of cognitive tests is required to unveil the effects of education on different domains of cognition. Aside from education, other environmental factors that have been linked to cognitive sex differences include lifestyle factors [73], socio-economic status (SES) [74, 75], and cultural differences [76, 77]. Of these, SES has been proposed to be a factor in the discrepancy of sex difference in cognitive aging [75]. However, such factors were not investigated in the current study. To further understand the connections between environmental factors and cognitive sex differences, additional research with considering more factors related to changing sex discrepancy in cognitive function is required.

At the molecular level, the present study provided preliminary evidence which suggested that cholinergic signaling may mediate the effect of education on attenuating cognitive sex differences. Acetylcholine (ACh) is thought to be a neuromodulator in the central nervous system in adults, altering neuronal excitability, inducing synaptic plasticity, and coordinating the firing of groups of neurons [78]. For example, the firing of dopamine (DA) neurons located in the ventral tegmental area, as well as the release of DA by their projections in the striatal area, is tightly controlled by the cholinergic pathway mediated by nicotinic acetylcholine receptors (nAChRs) [79]. DA depletion has been linked to widespread impairment in connectivity between frontal cortex and striatum as well as worsened set-shifting ability during WCST [80], and this ability in WCST indicates the metric of learning, abstract reasoning, and problem solving; participants with better set-shifting have a better opportunity of achieving a higher correct rate [81]. Furthermore, earlier research found that experience alters the cholinergic pathway in the hippocampus of mice by interaction with a gene product and repeated learning specifically amplified the effect of such gene product expression on this cholinergic projection pathway [82]. Effects of cholinergic enhancement on experience-dependent plasticity have also been observed in healthy adult auditory cortex [83, 84], as well as their visual perceptual learning [85], and a subsequent follow-up study elucidated that pharmacological cholinergic enhancement on visual perceptual learning is long-lasting [86]. Baskerville and collaborators [87], on the other hand, investigated the effects of ACh input depletion from nucleus basalis on experience-dependent plasticity in the cortex of young adult male rats and discovered that cholinergic-depleted animals showed no significant plasticity response. This suggested that a cholinergic enhancement on learning-dependent plasticity is likely to be sex-specific, with females benefiting more.

Another finding of the present study is that nearly half of the DPEs interacted directly with estrogen. Not surprisingly, the cholinergic pathways are critical sites for estrogen in the brain [88], and multiple basic and preclinical research over the past decades has clearly demonstrated that basal forebrain cholinergic systems rely upon estradiol support for adequate functioning [89], and these cholinergic projections play an essential role in attentional processes and learning [90, 91]. Besides this, available evidence suggests many of the effects of estrogen on neuronal function and plasticity, as well as cognitive performance, are related to or dependent on interactions with such cholinergic projections [92–95]. Consistent with these findings, we detected an estrogen-nAChR3 interaction that is associated with cognitive performance; nAChR3 expression was upregulated in females and this upregulation was more apparent in the secondary and tertiary education groups.

Although the cholinergic-estrogen interaction offers evidence for the effect of education on attenuating cognitive sex differences, other inherent or external factors with similar effects cannot be ignored. Prior research indicated that in rats, the arginine vasopressin (AVP) deficiency caused by a mutation in the *Avp* gene eliminated sex differences in the extinction of conditioned taste aversion [96] and social reinforcement [97]. Except for education, one socio-cognitive factor was also proposed to abolish sex differences in mental rotation performance [98], the probable underlying mechanism might be confidence as a mediator by which sex stereotype exerts its influence. That is, when females are the target of sex stereotype, their working memory capacity decreased dramatically [99, 100], resulting in a drop in logical reasoning [101] and mathematical performance [102].

There are some limitations to our study. First, this study included subjects ranging from young adults to elderly, which could have increased the variability of the sample. Furthermore, the proportion of middle-aged and elderly subjects was much higher than that of young adults, with the young participants clustering in the higher education group. A further study based on young adults with a larger sample size is needed to replicate the current findings. Second, cognitive function and education are related, but not only because education influences cognition; higher intellectual function can lead to more education. Third, except for age, the other confounders such as socioeconomic status and occupation were not controlled for in this study, despite the fact that both potentially contribute to the sex difference in cognitive performance. Fourth, although a computerized version of the WCST was utilized in the study, the response time of the participants has not been examined, which could be a more sensitive measure to show the sex-related differences, and future research that measures both WCST sub-scores and response time is needed to better understand the cognitive sex differences in WCST. Fifth, in this study, neuronal acetylcholine receptor subunit alpha-3 (nAChR3) was detected in serum of the research subjects; however, due to the brain-blood barrier, the majority of the nAChRs may be synthesized locally; therefore, further research into the change of nAChRs expression levels in brain tissues induced by learning is required to better understand these results. Sixth, the cross-sectional design of this study makes drawing causal inferences about the relationships among the variables under investigation challenging. Longitudinal studies are needed in the future to track changes in cognitive performance and protein expression over time and to assess potential causal relationships. Last, owing to the moderate sample size, further generalizing the results of our study needs to be cautious.

Conclusion

This study showed a preliminary connection between a cholinergic-estrogen interaction and the compensating effects of education on cognitive sex differences in a Thai healthy population. This suggested that learning experience is mediated by sex-dependent neural activity.

Education, for example, is associated with a differential modification of the cholinergic signaling in males and females, with women benefiting more. Once again, this implies that brain development is a result of genetic-environmental interaction, and future longitudinal research that takes into account more environmental factors such as lifestyle factors and medical conditions will be required to better understanding the environmental-biological mechanisms underlying cognitive sex performance.

Supporting information

S1 Table. Results of Wisconsin Card Sorting Test.
(XLSX)

S2 Table. Intensity of 11 differentially expressed proteins.
(XLSX)

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References

1. Cosgrove KP, Mazure CM, Staley JK. Evolving knowledge of sex differences in brain structure, function, and chemistry. Biol Psychiatry. 2007; 62(8):847–55. https://doi.org/10.1016/j.biopsych.2007.03.001 PMID: 17544382

2. Allen LS, Richey MF, Chai YM, Gorski RA. Sex differences in the corpus callosum of the living human being. J Neurosci. 1991; 11(4):933–42. https://doi.org/10.1523/JNEUROSCI.11-04-00933.1991 PMID: 2010816

3. Ingalhalikar M, Smith A, Parker D, Satterthwaite TD, Elliott MA, Ruparel K, et al. Sex differences in the structural connectome of the human brain. Proc Natl Acad Sci U S A. 2014; 111(2):823–8. https://doi.org/10.1073/pnas.1316909110 PMID: 24297904

4. Lenroot RK, Giedd JN. The changing impact of genes and environment on brain development during childhood and adolescence: initial findings from a neuroimaging study of pediatric twins. Dev Psychopathol. 2008; 20(4):1161–75. https://doi.org/10.1017/S0954579408000552 PMID: 18838036

5. Hanson JL, Chandra A, Wolfe BL, Pollak SD. Association between income and the hippocampus. PLoS One. 2011; 6(5):e18712. https://doi.org/10.1371/journal.pone.0018712 PMID: 21573231

6. Raizada RD, Richards TL, Meltzoff A, Kuhl PK. Socioeconomic status predicts hemispheric specialisation of the left inferior frontal gyrus in young children. Neuroimage. 2008; 40(3):1392–401. https://doi.org/10.1016/j.neuroimage.2008.01.021 PMID: 18308588

7. Kim J, Chey J, Kim SE, Kim H. The effect of education on regional brain metabolism and its functional connectivity in an aged population utilizing positron emission tomography. Neurosci Res. 2015; 94:50–61. https://doi.org/10.1016/j.neures.2014.12.009 PMID: 25557900

8. Panda R, Bharath RD, George L, Kanungo S, Reddy RP, Upadhyay N, et al. Unraveling Brain Functional Connectivity of encoding and retrieval in the context of education. Brain Cogn. 2014; 86:75–81. https://doi.org/10.1016/j.bandc.2014.01.018 PMID: 24568865

9. Perry A, Wen W, Kochan NA, Thalamuthu A, Sachdev PS, Breakspear M. The independent influences of age and education on functional brain networks and cognition in healthy older adults. Hum Brain Mapp. 2017; 38(10):5094–114. https://doi.org/10.1002/hbm.23717 PMID: 28685910

10. Boller B, Mellah S, Ducharme-Laliberte G, Belleville S. Relationships between years of education, regional grey matter volumes, and working memory-related brain activity in healthy older adults. Brain Imaging Behav. 2017; 11(2):304–17. https://doi.org/10.1007/s11682-016-9621-7 PMID: 27734304

11. Farfel JM, Nitrini R, Suemoto CK, Grinberg LT, Ferretti RE, Leite RE, et al. Very low levels of education and cognitive reserve: a clinicopathologic study. Neurology. 2013; 81(7):650–7. https://doi.org/10.1212/WNL.0b013e3182a08f1b PMID: 23873971

12. Abraham A. Gender and creativity: an overview of psychological and neuroscientific literature. Brain Imaging Behav. 2016; 10(2):609–18. https://doi.org/10.1007/s11682-015-9410-8 PMID: 26051636

13. Wehrwein EA, Lujan HL, DiCarlo SE. Gender differences in learning style preferences among undergraduate physiology students. Adv Physiol Educ. 2007; 31(2):153–7. https://doi.org/10.1152/advan.00060.2006 PMID: 17562903

14. Bakewell C, Mitchell V-W. Male versus female consumer decision making styles. Journal of Business Research. 2006; 59(12):1297–300.

15. Feizpour A, Parkington HC, Mansouri FA. Cognitive sex differences in effects of music in Wisconsin Card Sorting Test. Psychology of Music. 2018; 48(2):252–65.

16. Boone KB, Ghaffarian S, Lesser IM, Hill-Gutierrez E, Berman NG. Wisconsin Card Sorting Test performance in healthy, older adults: relationship to age, sex, education, and IQ. J Clin Psychol. 1993; 49(1):54–60. https://doi.org/10.1002/1097-4679(199301)49:1<54::aid-jclp2270490108>3.0.co;2-6 PMID: 8425935

17. Cinciute S, Daktariunas A, Ruksenas O. Hemodynamic effects of sex and handedness on the Wisconsin Card Sorting Test: the contradiction between neuroimaging and behavioural results. PeerJ. 2018; 6:e5890. https://doi.org/10.7717/peerj.5890 PMID: 30498629

18. Bloomberg M, Dugravot A, Dumurgier J, Kivimaki M, Fayosse A, Steptoe A, et al. Sex differences and the role of education in cognitive ageing: analysis of two UK-based prospective cohort studies. Lancet Public Health. 2021; 6(2):e106–e15. https://doi.org/10.1016/S2468-2667(20)30258-9 PMID: 33516287

19. Kopell NJ, Gritton HJ, Whittington MA, Kramer MA. Beyond the connectome: the dynome. Neuron. 2014; 83(6):1319–28. https://doi.org/10.1016/j.neuron.2014.08.016 PMID: 25233314

20. Medaglia JD, Lynall ME, Bassett DS. Cognitive network neuroscience. J Cogn Neurosci. 2015; 27(8):1471–91. https://doi.org/10.1162/jocn_a_00810 PMID: 25803596

21. Markham JA, Greenough WT. Experience-driven brain plasticity: beyond the synapse. Neuron Glia Biol. 2004; 1(4):351–63. https://doi.org/10.1017/s1740925x05000219 PMID: 16921405

22. Khanthiyong B, Thanoi S, Reynolds GP, Nudmamud-Thanoi S. Association study of the functional Catechol-O-Methyltranferase (COMT) Val(158)Met polymorphism on executive cognitive function in a Thai sample. Int J Med Sci. 2019; 16(11):1461–5. https://doi.org/10.7150/ijms.35789 PMID: 31673237

23. Freedman ML, Reich D, Penney KL, McDonald GJ, Mignault AA, Patterson N, et al. Assessing the impact of population stratification on genetic association studies. Nat Genet. 2004; 36(4):388–93. https://doi.org/10.1038/ng1333 PMID: 15052270

24. Yuan P, Raz N. Prefrontal cortex and executive functions in healthy adults: a meta-analysis of structural neuroimaging studies. Neurosci Biobehav Rev. 2014; 42:180–92. https://doi.org/10.1016/j.neubiorev.2014.02.005 PMID: 24568942

25. Nyhus E, Barcelo F. The Wisconsin Card Sorting Test and the cognitive assessment of prefrontal executive functions: a critical update. Brain Cogn. 2009; 71(3):437–51. https://doi.org/10.1016/j.bandc.2009.03.005 PMID: 19375839

26. Pineda D, Ardila A, Rosselli M, Cadavid C, Mancheno S, Mejia S. Executive dysfunctions in children with attention deficit hyperactivity disorder. Int J Neurosci. 1998; 96(3–4):177–96. https://doi.org/10.3109/00207459808986466 PMID: 10069618

27. Rybakowski JK, Borkowska A, Czerski PM, Kapelski P, Dmitrzak-Weglarz M, Hauser J. An association study of dopamine receptors polymorphisms and the Wisconsin Card Sorting Test in schizophrenia. J

Neural Transm (Vienna). 2005; 112(11):1575–82. https://doi.org/10.1007/s00702-005-0292-6 PMID: 15785860

28. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with the Folin phenol reagent. J Biol Chem. 1951; 193(1):265–75. PMID: 14907713

29. Tyanova S, Temu T, Cox J. The MaxQuant computational platform for mass spectrometry-based shot-gun proteomics. Nat Protoc. 2016; 11(12):2301–19. https://doi.org/10.1038/nprot.2016.136 PMID: 27809316

30. Tyanova S, Temu T, Sinitcyn P, Carlson A, Hein MY, Geiger T, et al. The Perseus computational plat-form for comprehensive analysis of (prote)omics data. Nat Methods. 2016; 13(9):731–40. https://doi.org/10.1038/nmeth.3901 PMID: 27348712

31. Bardou P, Mariette J, Escudie F, Djemiel C, Klopp C. jvenn: an interactive Venn diagram viewer. BMC Bioinformatics. 2014; 15(1):293. https://doi.org/10.1186/1471-2105-15-293 PMID: 25176396

32. Smyth GK. limma: Linear Models for Microarray Data. In: Gentleman R, Carey VJ, Huber W, Irizarry RA, Dudoit S, editors. Bioinformatics and Computational Biology Solutions Using R and Bioconductor. Statistics for Biology and Health. New York, NY: Springer New York; 2005. p. 397–420.

33. Chu VT, Gottardo R, Raftery AE, Bumgarner RE, Yeung KY. MeV+R: using MeV as a graphical user interface for Bioconductor applications in microarray analysis. Genome Biol. 2008; 9(7):R118. https://doi.org/10.1186/gb-2008-9-7-r118 PMID: 18652698

34. Ryu DY, Rahman MS, Pang MG. Determination of Highly Sensitive Biological Cell Model Systems to Screen BPA-Related Health Hazards Using Pathway Studio. Int J Mol Sci. 2017; 18(9). https://doi.org/10.3390/ijms18091909 PMID: 28878155

35. Nikitin A, Egorov S, Daraselia N, Mazo I. Pathway studio—the analysis and navigation of molecular networks. Bioinformatics. 2003; 19(16):2155–7. https://doi.org/10.1093/bioinformatics/btg290 PMID: 14594725

36. Fife D. The Eight Steps of Data Analysis: A Graphical Framework to Promote Sound Statistical Analy-sis. Perspect Psychol Sci. 2020; 15(4):1054–75. https://doi.org/10.1177/1745691620917333 PMID: 32502366

37. Liang F, Xiong M. Bayesian detection of causal rare variants under posterior consistency. PLoS One. 2013; 8(7):e69633. https://doi.org/10.1371/journal.pone.0069633 PMID: 23922764

38. Dienes Z, Coulton S, Heather N. Using Bayes factors to evaluate evidence for no effect: examples from the SIPS project. Addiction. 2018; 113(2):240–6. https://doi.org/10.1111/add.14002 PMID: 28804980

39. Cinelli C, Hazlett C. Making Sense of Sensitivity: Extending Omitted Variable Bias. Journal of the Royal Statistical Society Series B: Statistical Methodology. 2020; 82(1):39–67.

40. Rouder JN, Morey RD. Default Bayes Factors for Model Selection in Regression. Multivariate Behav Res. 2012; 47(6):877–903. https://doi.org/10.1080/00273171.2012.734737 PMID: 26735007

41. Leppink J, O'Sullivan P, Winston K. Effect size—large, medium, and small. Perspect Med Educ. 2016; 5(6):347–9. https://doi.org/10.1007/s40037-016-0308-y PMID: 27752936

42. Lung FW, Chen PF, Shu BC. Performance of Wisconsin Card Sorting Test in five-year-old children in Taiwan: Relationship to intelligence and cognitive development. PLoS One. 2018; 13(8):e0202099. https://doi.org/10.1371/journal.pone.0202099 PMID: 30161263

43. Nagahama Y, Fukuyama H, Yamauchi H, Katsumi Y, Magata Y, Shibasaki H, et al. Age-related changes in cerebral blood flow activation during a Card Sorting Test. Exp Brain Res. 1997; 114 (3):571–7. https://doi.org/10.1007/pl00005665 PMID: 9187292

44. Lie CH, Specht K, Marshall JC, Fink GR. Using fMRI to decompose the neural processes underlying the Wisconsin Card Sorting Test. Neuroimage. 2006; 30(3):1038–49. https://doi.org/10.1016/j.neuroimage.2005.10.031 PMID: 16414280

45. Nagahama Y, Sadato N, Yamauchi H, Katsumi Y, Hayashi T, Fukuyama H, et al. Neural activity during attention shifts between object features. Neuroreport. 1998; 9(11):2633–8. https://doi.org/10.1097/00001756-199808030-00038 PMID: 9721946

46. Horner MD, Flashman LA, Freides D, Epstein CM, Bakay RA. Temporal lobe epilepsy and perfor-mance on the Wisconsin Card Sorting Test. J Clin Exp Neuropsychol. 1996; 18(2):310–3. https://doi.org/10.1080/01688639608408285 PMID: 8780965

47. Nelson HE. A modified card sorting test sensitive to frontal lobe defects. Cortex. 1976; 12(4):313–24. https://doi.org/10.1016/s0010-9452(76)80035-4 PMID: 1009768

48. Lawton CA. Gender and Regional Differences in Spatial Referents Used in Direction Giving. Sex Roles. 2001; 44(5/6):321–37.

49. Ward SL, Newcombe N, Overton WF. Turn Left at the Church, Or Three Miles North. Environment and Behavior. 2016; 18(2):192–213.

50. Feng J, Spence I, Pratt J. Playing an action video game reduces gender differences in spatial cognition. Psychol Sci. 2007; 18(10):850–5. https://doi.org/10.1111/j.1467-9280.2007.01990.x PMID: 17894600

51. Cox SR, Dickie DA, Ritchie SJ, Karama S, Pattie A, Royle NA, et al. Associations between education and brain structure at age 73 years, adjusted for age 11 IQ. Neurology. 2016; 87(17):1820–6. https://doi.org/10.1212/WNL.0000000000003247 PMID: 27664981

52. Lee D, Kwak S, Chey J. Parallel Changes in Cognitive Function and Gray Matter Volume After Multi-Component Training of Cognitive Control (MTCC) in Adolescents. Front Hum Neurosci. 2019; 13:246. https://doi.org/10.3389/fnhum.2019.00246 PMID: 31379541

53. Rzezak P, Squarzoni P, Duran FL, de Toledo Ferraz Alves T, Tamashiro-Duran J, Bottino CM, et al. Relationship between Brain Age-Related Reduction in Gray Matter and Educational Attainment. PLoS One. 2015; 10(10):e0140945. https://doi.org/10.1371/journal.pone.0140945 PMID: 26474472

54. Davis CL, Tomporowski PD, McDowell JE, Austin BP, Miller PH, Yanasak NE, et al. Exercise improves executive function and achievement and alters brain activation in overweight children: a randomized, controlled trial. Health Psychol. 2011; 30(1):91–8. https://doi.org/10.1037/a0021766 PMID: 21299297

55. Diamond A. Activities and Programs That Improve Children's Executive Functions. Curr Dir Psychol Sci. 2012; 21(5):335–41. https://doi.org/10.1177/0963721412453722 PMID: 25328287

56. Moreno S, Bialystok E, Barac R, Schellenberg EG, Cepeda NJ, Chau T. Short-term music training enhances verbal intelligence and executive function. Psychol Sci. 2011; 22(11):1425–33. https://doi.org/10.1177/0956797611416999 PMID: 21969312

57. Peterson DA, Thaut MH. Music increases frontal EEG coherence during verbal learning. Neurosci Lett. 2007; 412(3):217–21. https://doi.org/10.1016/j.neulet.2006.10.057 PMID: 17178191

58. Wilmsmeier A, Ohrmann P, Suslow T, Siegmund A, Koelkebeck K, Rothermundt M, et al. Neural correlates of set-shifting: decomposing executive functions in schizophrenia. J Psychiatry Neurosci. 2010; 35(5):321–9. https://doi.org/10.1503/jpn.090181 PMID: 20731964

59. Chen C, Khanthiyong B, Thaweetee-Sukjai B, Charoenlappanit S, Roytrakul S, Thanoi S, et al. A proteomic study indicating susceptibility to excitotoxicity induced by the N-methyl-D- aspartate receptor hyperfunction as a potential mechanism underlying differences in cognitive function between males and females in a Thai healthy population. PREPRINT (Version 1) available at Research Square. 2023.

60. Burstein SR, Kim HJ, Fels JA, Qian L, Zhang S, Zhou P, et al. Estrogen receptor beta modulates permeability transition in brain mitochondria. Biochim Biophys Acta Bioenerg. 2018; 1859(6):423–33. https://doi.org/10.1016/j.bbabio.2018.03.006 PMID: 29550215

61. Mendelowitsch A, Ritz MF, Ros J, Langemann H, Gratzl O. 17beta-Estradiol reduces cortical lesion size in the glutamate excitotoxicity model by enhancing extracellular lactate: a new neuroprotective pathway. Brain Res. 2001; 901(1–2):230–6. https://doi.org/10.1016/s0006-8993(01)02359-9 PMID: 11368971

62. Zhao L, Brinton RD. Estrogen receptor alpha and beta differentially regulate intracellular Ca(2+) dynamics leading to ERK phosphorylation and estrogen neuroprotection in hippocampal neurons. Brain Res. 2007; 1172:48–59. https://doi.org/10.1016/j.brainres.2007.06.092 PMID: 17803971

63. Eling P, Derckx K, Maes R. On the historical and conceptual background of the Wisconsin Card Sorting Test. Brain Cogn. 2008; 67(3):247–53. https://doi.org/10.1016/j.bandc.2008.01.006 PMID: 18328609

64. Hsieh PC, Yeh TL, Lee IH, Huang HC, Chen PS, Yang YK, et al. Correlation between errors on the Wisconsin Card Sorting Test and the availability of striatal dopamine transporters in healthy volunteers. J Psychiatry Neurosci. 2010; 35(2):90–4. https://doi.org/10.1503/jpn.090007 PMID: 20184806

65. Dempster K, Norman R, Theberge J, Densmore M, Schaefer B, Williamson P. Cognitive performance is associated with gray matter decline in first-episode psychosis. Psychiatry Res Neuroimaging. 2017; 264:46–51. https://doi.org/10.1016/j.pscychresns.2017.04.007 PMID: 28458083

66. Vasic N, Walter H, Hose A, Wolf RC. Gray matter reduction associated with psychopathology and cognitive dysfunction in unipolar depression: a voxel-based morphometry study. J Affect Disord. 2008; 109(1–2):107–16. https://doi.org/10.1016/j.jad.2007.11.011 PMID: 18191459

67. Gennatas ED, Avants BB, Wolf DH, Satterthwaite TD, Ruparel K, Ciric R, et al. Age-Related Effects and Sex Differences in Gray Matter Density, Volume, Mass, and Cortical Thickness from Childhood to Young Adulthood. J Neurosci. 2017; 37(20):5065–73. https://doi.org/10.1523/JNEUROSCI.3550-16.2017 PMID: 28432144

68. Halpern DF, Benbow CP, Geary DC, Gur RC, Hyde JS, Gernsbacher MA. The Science of Sex Differences in Science and Mathematics. Psychol Sci Public Interest. 2007; 8(1):1–51. https://doi.org/10.1111/j.1529-1006.2007.00032.x PMID: 25530726

69. Davis T, Goldwater M, Giron J. From Concrete Examples to Abstract Relations: The Rostrolateral Prefrontal Cortex Integrates Novel Examples into Relational Categories. Cereb Cortex. 2017; 27(4):2652–70. https://doi.org/10.1093/cercor/bhw099 PMID: 27130661

70. O'Bryan SR, Worthy DA, Livesey EJ, Davis T. Model-based fMRI reveals dissimilarity processes underlying base rate neglect. Elife. 2018; 7. https://doi.org/10.7554/eLife.36395 PMID: 30074478

71. Kalia V, Vishwanath K, Knauft K, Vellen BV, Luebbe A, Williams A. Acute Stress Attenuates Cognitive Flexibility in Males Only: An fNIRS Examination. Front Psychol. 2018; 9:2084. https://doi.org/10.3389/fpsyg.2018.02084 PMID: 30443232

72. Miranda AR, Franchetto Sierra J, Martinez Roulet A, Rivadero L, Serra SV, Soria EA. Age, education and gender effects on Wisconsin card sorting test: standardization, reliability and validity in healthy Argentinian adults. Neuropsychol Dev Cogn B Aging Neuropsychol Cogn. 2020; 27(6):807–25. https://doi.org/10.1080/13825585.2019.1693491 PMID: 31744387

73. Wang J, Xiao LD, Wang K, Luo Y, Li X. Gender Differences in Cognitive Impairment among Rural Elderly in China. International Journal of Environmental Research and Public Health. 2020; 17 (10):3724. https://doi.org/10.3390/ijerph17103724 PMID: 32466167

74. Liu SZ, Tahmasebi G, Sheng Y, Dinov ID, Tsilimingras D, Liu X. Sex difference in the associations of socioeconomic status, cognitive function and brain volume with dementia in old adults: Findings from the OASIS study. medRxiv. 2023. https://doi.org/10.1101/2023.01.05.23284240 PMID: 36711777

75. Jin Y, Hong C, Luo Y. Sex differences in cognitive aging and the role of socioeconomic status: Evidence from multi-cohort studies. Psychiatry Res. 2023; 321:115049. https://doi.org/10.1016/j.psychres.2023.115049 PMID: 36706558

76. Mann VA, Sasanuma S, Sakuma N, Masaki S. Sex differences in cognitive abilities: a cross-cultural perspective. Neuropsychologia. 1990; 28(10):1063–77. https://doi.org/10.1016/0028-3932(90)90141-a PMID: 2267058

77. Reilly D. Gender, culture, and sex-typed cognitive abilities. PLoS One. 2012; 7(7):e39904. https://doi.org/10.1371/journal.pone.0039904 PMID: 22808072

78. Picciotto MR, Higley MJ, Mineur YS. Acetylcholine as a neuromodulator: cholinergic signaling shapes nervous system function and behavior. Neuron. 2012; 76(1):116–29. https://doi.org/10.1016/j.neuron.2012.08.036 PMID: 23040810

79. Dong Y, Dani JA, Blakely RD. Choline transporter hemizygosity results in diminished basal extracellular dopamine levels in nucleus accumbens and blunts dopamine elevations following cocaine or nicotine. Biochem Pharmacol. 2013; 86(8):1084–8. https://doi.org/10.1016/j.bcp.2013.07.019 PMID: 23939187

80. Nagano-Saito A, Leyton M, Monchi O, Goldberg YK, He Y, Dagher A. Dopamine depletion impairs frontostriatal functional connectivity during a set-shifting task. J Neurosci. 2008; 28(14):3697–706. https://doi.org/10.1523/JNEUROSCI.3921-07.2008 PMID: 18385328

81. Teubner-Rhodes S, Vaden KI Jr., Dubno JR, Eckert MA. Cognitive persistence: Development and validation of a novel measure from the Wisconsin Card Sorting Test. Neuropsychologia. 2017; 102:95–108. https://doi.org/10.1016/j.neuropsychologia.2017.05.027 PMID: 28552783

82. Brooks AI, Cory-Slechta DA, Federoff HJ. Gene-experience interaction alters the cholinergic septohippocampal pathway of mice. Proc Natl Acad Sci U S A. 2000; 97(24):13378–83. https://doi.org/10.1073/pnas.230169397 PMID: 11078505

83. Thiel CM, Friston KJ, Dolan RJ. Cholinergic modulation of experience-dependent plasticity in human auditory cortex. Neuron. 2002; 35(3):567–74. https://doi.org/10.1016/s0896-6273(02)00801-2 PMID: 12165477

84. Thiel CM, Bentley P, Dolan RJ. Effects of cholinergic enhancement on conditioning-related responses in human auditory cortex. Eur J Neurosci. 2002; 16(11):2199–206. https://doi.org/10.1046/j.1460-9568.2002.02272.x PMID: 12473087

85. Rokem A, Silver MA. Cholinergic enhancement augments magnitude and specificity of visual perceptual learning in healthy humans. Curr Biol. 2010; 20(19):1723–8. https://doi.org/10.1016/j.cub.2010.08.027 PMID: 20850321

86. Rokem A, Silver MA. The benefits of cholinergic enhancement during perceptual learning are long-lasting. Front Comput Neurosci. 2013; 7:66. https://doi.org/10.3389/fncom.2013.00066 PMID: 23755006

87. Baskerville KA, Schweitzer JB, Herron P. Effects of cholinergic depletion on experience-dependent plasticity in the cortex of the rat. Neuroscience. 1997; 80(4):1159–69. https://doi.org/10.1016/s0306-4522(97)00064-x PMID: 9284068

88. Newhouse P, Dumas J. Estrogen-cholinergic interactions: Implications for cognitive aging. Horm Behav. 2015; 74:173–85. https://doi.org/10.1016/j.yhbeh.2015.06.022 PMID: 26187712

89. Gibbs RB. Estrogen therapy and cognition: a review of the cholinergic hypothesis. Endocr Rev. 2010; 31(2):224–53. https://doi.org/10.1210/er.2009-0036 PMID: 20019127

90. Sarter M, Hasselmo ME, Bruno JP, Givens B. Unraveling the attentional functions of cortical cholinergic inputs: interactions between signal-driven and cognitive modulation of signal detection. Brain Res Brain Res Rev. 2005; 48(1):98–111. https://doi.org/10.1016/j.brainresrev.2004.08.006 PMID: 15708630

91. Everitt BJ, Robbins TW. Central cholinergic systems and cognition. Annu Rev Psychol. 1997; 48:649–84. https://doi.org/10.1146/annurev.psych.48.1.649 PMID: 9046571

92. Dumas J, Hancur-Bucci C, Naylor M, Sites C, Newhouse P. Estradiol interacts with the cholinergic system to affect verbal memory in postmenopausal women: evidence for the critical period hypothesis. Horm Behav. 2008; 53(1):159–69. https://doi.org/10.1016/j.yhbeh.2007.09.011 PMID: 17964576

93. Marriott LK, Korol DL. Short-term estrogen treatment in ovariectomized rats augments hippocampal acetylcholine release during place learning. Neurobiol Learn Mem. 2003; 80(3):315–22. https://doi.org/10.1016/j.nlm.2003.08.003 PMID: 14521873

94. Daniel JM, Dohanich GP. Acetylcholine mediates the estrogen-induced increase in NMDA receptor binding in CA1 of the hippocampus and the associated improvement in working memory. J Neurosci. 2001; 21(17):6949–56. https://doi.org/10.1523/JNEUROSCI.21-17-06949.2001 PMID: 11517282

95. Daniel JM, Hulst JL, Lee CD. Role of hippocampal M2 muscarinic receptors in the estrogen-induced enhancement of working memory. Neuroscience. 2005; 132(1):57–64. https://doi.org/10.1016/j.neuroscience.2005.01.002 PMID: 15780466

96. Brot MD, Bernstein IL, Dorsa DM. Vasopressin deficiency abolishes a sexually dimorphic behavior in Brattleboro rats. Physiol Behav. 1992; 51(4):839–43. https://doi.org/10.1016/0031-9384(92)90124-k PMID: 1594683

97. Schatz KC, Martin CD, Ishiwari K, George AM, Richards JB, Paul MJ. Mutation in the vasopressin gene eliminates the sex difference in social reinforcement in adolescent rats. Physiol Behav. 2019; 206:125–33. https://doi.org/10.1016/j.physbeh.2019.04.004 PMID: 30951747

98. Estes Z, Felker S. Confidence mediates the sex difference in mental rotation performance. Arch Sex Behav. 2012; 41(3):557–70. https://doi.org/10.1007/s10508-011-9875-5 PMID: 22130691

99. Beilock SL, Rydell RJ, McConnell AR. Stereotype threat and working memory: mechanisms, alleviation, and spillover. J Exp Psychol Gen. 2007; 136(2):256–76. https://doi.org/10.1037/0096-3445.136.2.256 PMID: 17500650

100. Rydell RJ, McConnell AR, Beilock SL. Multiple social identities and stereotype threat: imbalance, accessibility, and working memory. J Pers Soc Psychol. 2009; 96(5):949–66. https://doi.org/10.1037/a0014846 PMID: 19379029

101. Regner I, Smeding A, Gimmig D, Thinus-Blanc C, Monteil JM, Huguet P. Individual differences in working memory moderate stereotype-threat effects. Psychol Sci. 2010; 21(11):1646–8. https://doi.org/10.1177/0956797610386619 PMID: 20959509

102. Schmader T, Forbes CE, Zhang S, Mendes WB. A metacognitive perspective on the cognitive deficits experienced in intellectually threatening environments. Pers Soc Psychol Bull. 2009; 35(5):584–96. https://doi.org/10.1177/0146167208330450 PMID: 19228598