Cortisol response to the presence of same and opposite sex individuals in various social environments

by

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Abstract

Consistent with the Standard Social Science Model, educational researchers, more times than not, rely on explanations steeped in cultural transmission to describe and explain student behavior. The study of single vs. mixed-sex environments is no exception. Researchers who have categorically ignored the role of biology have dominated this debate. Nevertheless, every aspect of the human`s life has a biological foundation and unless it was biologically possible, it would not exist. Given the interplay of biological, psychological and sociological systems, it is unlikely that the topical intransigence surrounding the debate of single vs. mixed- sex environs will be resolved unless future investigations consider these elements. The present work is an initial step in such a direction.

Inspired by sexual selection theory and supported by bodies of neuroendocrinological research in humans and other animals, this study integrated the assumptions of evolutionary psychology that all intrasexual interactions serve reproductive purposes to some degree and investigated the organismal cortisol response to single and mixed- sex social environments with added cognitive task. Cortisol is the most potent glucocorticoid secreted by HPA-axis and in normal for individual concentrations enhances learning and memory formation, while elevated cortisol blocks necessary for learning chemical reactions in the brain.

Twenty participants were asked to complete a cognitive task in mixed and single sex environs. Saliva samples of five males and four females were randomly chosen and analyzed using ELISA kit to assess free cortisol level.

Results indicated that human cortisol response is sensitive to environmental sex composition. Participants' cortisol levels (both males and females) increased significantly in the presence of opposite- sex counterparts compared to levels in single- sex environs. Interestingly, when a cognitive task was added to both the single and mixed- sex environs, cortisol levels did not significantly change. That is, cortisol response in mixed- sex environs with a cognitive task was not significantly greater than cortisol response in single- sex environs with and without a cognitive task.

These findings are the first demonstration of cortisol response to the presence of same and opposite- sex counterparts in social groups with involvement of a cognitive task.

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CHAPTER 1

Single Sex vs. Coeducation

Although the debate over single- sex vs. mixed- sex groupings has obvious educational implications, it is noteworthy that the educational stakeholder's rationale for choosing one grouping over another has, more often than not, been an issue of politics and economics rather than biology in pedagogy (Kaminsky &Forbes, 2008; Mael, Alonso, Gibson, Rogers, & Smith, 2005; Tyack & Hansot, 1992). Prior to the twentieth century, formal education was decidedly single sex worldwide. Yet, by the early 1900s mixed- sex grouping (coeducation) throughout public schools was advanced through the promise of social equality. As compulsory education became the norm, and coeducation was in the economic interests of school districts (single- sex education required twice as many schools with twice as many teachers and twice as many support staff), coeducation became the standard model among public education institutions; meanwhile, single-sex education became the province of private, often religious schools, especially in the U.S. (Tyack & Hansot, 1992; Salomone, 2003). Yet, toward the end of the twentieth century, a renewed interest in single- sex education was evidenced in a number of countries (e.g. Australia, Great Britain, New Zealand, U.S) (Billger, 2009; Cable & Spradlin, 2008; Gurian, Stevens & Daniels, 2009; Meyer, 2008).

Current Trends and Limitations

A traditional argument of the utility of one approach over the other centers on the idea that separate is inherently unequal (coeducation) but avoids opposite sex distractions (single sex). At first glance, this is so commonsensical that it needs no further elaboration. Existing in the educational research claim is that single- sex high schools have more serious and studious climates, which are more immune to the dominant "rating and dating" culture of coeducational schools (Finn, 1980; Koepke, 1991; Lee & Bryk, 1986) which are portrayed as “jungles of dating and social maneuver” (Coleman, 1961; Goodlad, 1984) and in which overly aroused adolescents are “subjected to intense sexual distractions and competitions during the critical stages of their educations “ (Gilder, 1973, p. 210; Kolesnik, 1969). The primary premise of this argument is that in a single-sex school, students are afforded down time during which they do not have to be concerned about how they are perceived sexually. The “psychic energy” freed up by the separation of the sexes is presumed to be available and diverted to academic study. However, why youth freed from “rating and dating issues” will spend more time and energy on academics is unclear.

Additionally, supporters of single-sex education believe that separating students based on sex will lead to less gender stereotyping in the classroom, because without the opposite sex in the classroom, students will not be expected to follow specific gender norms. However, opponents of single- sex education fear that separating students by sex will actually increase gender stereotyping because separating students by sex is itself a form of gender stereotyping. They also note that the research supporting single-sex education is extremely limited (Carr, 2007), and not representative of the diversity of students in US schools, arguably biased by our society’s prevalent gender stereotypes.

More recently, another perspective loosely co-opts elements of socio-biology and the cognitive sciences, committed to the idea that males and females learn differently (single sex) but require co-mingling to optimize potential (coeducation). Proponents of single- sex education gender differences reflect underlying biological factors, including hormone levels, neurological function, and even hearing ability, (Gurian, 2001;Gurian, Stevens & Daniels, 2009; James, 2007; Sax,2005) ultimately suggesting that gender differences are the most significant differences among schoolchildren and that these differences can be better addressed in single-sex classrooms. The problem is that publications from popular authors citing neuroscientific evidence, (Biddulph, 2008; Gurian, 1996; Jensen, 1998, 2005; King & Gurian, 2006) more times than not, are at odds with primary neuroscience research and consumers seem all too ready to accept explanations that allude to neuroscience, even if they are not accurate reflections of the scientific data, and even if they would otherwise be seen as far less satisfying (Weisberg, Keil, Goodstein, Rawson & Gray, 2008). Halpern et al. (2011) called these explanations “pseudoscientific”, deeply misguided, and often justified by weak, cherry picked, or misconstrued scientific claims rather than by valid scientific evidence. In fact, research shows that the behavioral, psychological, and cognitive differences among the individual members of any group of girls or any group of boys are much greater, and more relevant from an instructional standpoint, than the differences between boys and girls as groups (Eliot, 2009).

Whatever the rationale as to the utility of single- sex education or coeducation, results are mixed. For every study that demonstrates an advantage of single sex education, another suggests that coeducation is preferable (See Mael et al., 2005; Riordan et al., 2008). Research evidence, although indicative that single- sex schools can benefit some students in some realms of academic and socio- emotional accomplishment, is equivocal and can be summarized as follows:

> The academic and developmental consequences of attending one type of school versus another type of school are virtually zero for middle-class and otherwise advantaged students; by contrast, the consequences are significant for students who are or have been historically or traditionally disadvantaged - minorities, low- and working-class youth, and females, so long as the females are not affluent (Riordan, 1994).

Regardless of the position researchers take, a number of limitations confront consumers of single- sex vs. coeducational literature. Many accounts are simply rational pleas based on anecdotal information (Eliot, 2011). What is more, no valid evidence establishes a causal connection between single-sex education in public primary and secondary schools and better educational outcomes (Mael, et al., 2005). Conclusions emerging from empirical investigation are, at best, correlational; the use of experimental methods is non-existent. Moreover, researchers tend to rely on distal outcomes, e.g., grade point average, standardized test scores, discipline referrals. Another concern is the operational definition of single-sex vs. co-education. When explicitly stated, most investigations solely consider the sex of students whereas others consider the sex of teachers and support staff, as well. Yet, the most severe limitation is the misguided attempts to decipher how sex is implicated in learning.

Statement of the Problem

Traditionally, researcher rationales for the utility of single or mixed- sex education reflect an environmental causation model, ignoring the biological foundations of human behavior. Cronk, Chagnon, and Irons (2000) comically refer to examples such as this as species chauvinism: a belief that among animals, humans alone have the power to elude troubles of biological predisposition. This is contrasted by more recent attempts by advocates of single sex education who suggest males and females require separate environments because of genetically fixed differences that produce thought and behavior in immutable, universal form.

Problem is, human forms and functions do not arise from the simple reading of a genetic blueprint anymore than environments write on a “blank slate.” As Silverman and Philips (1998) suggest, “the division between heredity and environment is spurious” (p. 607). There are no pure genetic or environmental effects nor is genetic and environmental potential additive (Bjorklund

& Bering, 2000; Scarr, 1992). Instead, human nature emerges via correlation and interaction between all levels of biological and experiential factors, from the genetic through the cultural. (Bjorklund & Bering, 2000).

Among researchers immune to the "Nature vs. Nature" debate, explanations of the "how" and "why" of human nature include two causes: distal and proximate. Distal explanations consider the evolutionary forces that produced *Homo sapiens* whereas proximate explanations consider how physiological mechanisms interact with environmental stimuli. As such, consideration of human thought and behavior should reflect both causes (Dretske, 2004). To date, however, there is no published study within the single- sex vs. coeducation literature that considers the distal and proximate mechanisms by which sex is implicated in learning.

Theoretical Framework

Distal Explanations of Sex as Environmental Stimulus

Though behavioral and social sciences have a long history of suggesting evolutionary processes are irrelevant to the study of human behavior because of the existence of culture, intelligence and learning, every aspect of a human life has an evolutionary foundation. "Human minds, human behaviour, human artefacts, and human culture are all biological (created by evolution) phenomena…" (Tooby & Cosmides, 1992). And though ontogeny of humans has been characterized by a remarkable expansion in consciousness, complex learning and culture - transmission mechanisms, analysis of these mechanisms must be executed in terms of evolutionary ends (DeVore & Tooby, 1987). Like other traits, the human mind, (capable of specific cognitive operations such as language, facial recognition and processing social interactions) is either an adaptation or by-product of natural and sexual selection that enables humans to survive and reproduce.

Though Darwin is universally linked with evolution, he was certainly not the first to consider it (i.e., Lamarck, Saint -Hilaire). He and Wallace were, however, the first to express evolution as operating through the heritability of population variance among traits created by natural selection. The principle of natural selection follows from their observations that, as all species are capable of overproducing offspring and resources for support of offspring is limited, a struggle for existence among individuals necessarily ensues. As individuals differ on traits (i.e. adaptations) that enable them to survive and reproduce, populations are will produce necessarily different offspring (Darwin, 1851; Mayr, 1962; Crawford, 1998). Via natural selection, species and environments evolve hand in hand. Thus, natural selection ensures a workable fit between living things and the environment in which they develop.

The fact that men and women are biologically different is readily agreed upon: XX = female, XY = male. This single universal genetic difference between a male human and a female human results in an almost identical phenotype: males and females share every organ - every muscle, every bone, and indeed every part of the brain - each serving the same function, interrelated to other organs in precisely the same manner - with qualitative sex dimorphism in only the tiny minority with exclusively procreative functions. Without regard for gender, genes encode phenotypes that are successful in obtaining healthy and regular supplies of water and food, in procreating, and in protection from disease, injuries, social or predator attack, accidents and errors of action.

Nevertheless, in humans, as in many other sexually reproducing species, individuals live under constant sexual selection pressure and frequently compete for the reproductive access to opposite-sex partners (Buss, 1988; Walters & Crawford, 1994). Indeed, intrasexual competition has been a central driving force behind human evolution (Darwin, 1851, 1871; Buss, 2007) with sexual selection often stronger than natural selection, as it frequently drives trait values beyond their naturally selected optima. This process has ample social dimensions, and because humans are highly social, there is a definite gender dimorphism in the mechanical and mental aptitudes of males and females. Every fertile human is biologically male or female and each brings decidedly different potential to the requirements of species propagation. Throughout evolutionary history, sexual selection pressures have differed to some degree for sexually dimorphic organisms; greatest among these are the conditions that gave rise to the traits that ensure conception, birth, and survival of offspring (Trivers, 1972; Buss, 1994; Geary, 1999). Biologically, the costs associated with mating differ for each sex, with greater costs attributed to females. Take conception among humans during the EEA (Environment of Evolutionary Adaptedness), for example, where female investiture is a bit more complicated than a male’s. Those females assume that their reproductive goals (as measured by the number of viable offspring born) are best met through parental effort. Consequently, intersexual choice will be greater among females than males. Male reproductive efforts, however, are best met by mating effort. The number of males pursuing females is greater than the number of females pursuing males which suggests intrasexual competition will be greater for males than females (Trivers, 1972, 1985; Buss, 1994).

Reproductive effort in modern humans is significantly more complicated. Most notable is the difference in parental effort among males and mate selection among females from EEA to modernity. Though females still disproportionately provide parental effort and males have more sexual partners than females, it is fundamentally inaccurate to suggest that males will never demonstrate intersexual choice nor females demonstrate intrasexual competition. Instead, the difference is a matter of degree and largely reflective of cultural variations in marriage systems (Geary, 1998).

Of course, most differences between males and females we see today are not directly related to intersexual choice and intrasexual competition of either sex during EEA. Instead, most are secondary sexual characteristics—emerging as co-opted physical, behavioral and cognitive, differences that originally evolved via natural selection since features that evolved under sexual selection pressure typically are not seen until the organism is able to mate. The fact that most of these differences do not occur in infants and children, but are typical primarily in the adult stage, indicates that many of the effects are the result of hormonal events occurring at puberty (Beach, 1974). Like the primary sexual characteristics (i.e. differences in external genitalia), secondary sexual characteristics are largely controlled or mediated by X-linked genes (Rice, 1984), although they differ in control from the primary ones in that the *environment* has a more direct influence on their expression. For the ongoing debate on single-sex vs. mixed- sex education, this fact is instructive.

Proximal Explanations of Sex as Environmental Stimulus

As stated above, proximate explanations consider how physiological mechanisms interact with environmental stimuli to produce human responses. In consideration of single-sex vs. coeducation, recall Wiener's (1948) assertion that mental events are structured informational manipulations of physical systems in the brain, if sex of an individual fails to activate specific physical systems—evidenced through measureable physiological response(s) in the brain of another individual—researchers cannot empirically state that sex rises to the level of a relevant environmental stimulus for the first individual. If there is a measurable response then the activated physical system is implicated, at least in part, as a proximal mechanistic cause of sex as a relevant environmental stimulus.

It is important to note that despite the contention that evolutionary processes are involved in the production of proximal mechanisms, proximal outcomes can be seen as either the product of an adaptive brain mechanism specialized to produce that outcome or as the by-product of an adaptation (or adaptations) not specially designed to produce the outcome in question. Regarding the evaluation of sex as an environmental stimulus this distinction leaves researchers the task of distinguishing outcomes that are produced by specialized design (e.g., potential mate detection adaptation) from those that are the by-products of adaptations designed for other purposes (e.g. a learned response to sex as an environmental stimulus via operant conditioning).

Specification of potential, precise proximate mechanisms involved in sex as an environmental stimulus begins with an examination of the nervous and endocrine systems, as both are centrally involved in the production and coordination of human response. The primary interface between the central nervous system (CNS) and the endocrine system is the hypothalamus, which is at the same time an integral part of CNS and an endocrine gland. The hypothalamus serves as the assimilating center for interoceptive (originating within the CNS) and exteroceptive (originating outside the CNS) stimuli. This diverse collection of nuclei at the base of the brain processes the stimuli and generates appropriate responses mediated in large part by the neuroendocrine and endocrine signals originated along the hypothalamo-pituitary axis (HPA). Glucocorticoids produced by HPA help modulate an organism's adaptive response to a variety of physiological or psychosocial stressors (Sapolsky, 1992). The HPA axis mainly controls levels of glucocorticoids, with cortisol being the most potent glucocorticoid secreted in response to activation of HPA. Traditionally cortisol has been seen as the body's "stress system", although new research is beginning to show that the HPA axis should instead be thought of as the body's energy regulator, as it is ultimately responsible for controlling virtually all of the hormones, nervous system activity, and regulation of many bodily processes and energy storage and expenditure (Roney et al., 2007; Flinn, 2006).

Cortisol response to various environmental stimuli possesses particular relevance to educators as the role of glucocorticoids in sustaining and facilitating cognitive functions is well documented and supported by subjective reports and behavioral observations. Based on the available data, it is evident that both endogenous and exogenous based increases in glucocorticoids are associated with deficits in both memory and attention (Reus et al., 1985; Lupien & Forget, 1995). Secondly, evidence that glucocorticoids can compromise the functioning of the hippocampus (Meaney et al., 1995), has led investigators to speculate that hippocampal-based cognitive functions (such as consolidation of information from short-term memory to long-term memory and spatial navigation) may be at particular risk from the deleterious effects of glucocorticoids (Lupien and Forget, 1995; Wolkowitz et al., 1997). Finally, in their review of the human and animal literature on the acute effects of glucocorticoids, Lupien and McEwen (1997) concluded that an inverted U-shaped relationship is evident between glucocorticoids and the nature and magnitude of cognitive dysfunction.

For those concerned with the utility of single- sex vs. coeducation and proposing that the sex of students is the driving force behind success or failure of such arrangements, endocrine response to sex as environmental stimulus is paramount. Traditionally, because hormones have powerful effects on the nervous system and behavior throughout the life of an individual, unidirectional relationship between hormone secretion and behavior form the focus of investigation. Lately though, the bidirectional nature of this relationship is emphasized with focus on the functionality of endocrine changes driven by environmental influences (van Anders, &Watson, 2006). The development of salivary steroid immunoassay has greatly simplified the inclusion of endocrine variables in bio-behavioral research. Sex steroids are of particular relevance to research into sexually selected behaviors and can be reliably measured in saliva. Examining how behavioral influences affect hormones, and how this plays back onto behavior, yielded quite testable hypotheses in many areas of the behavioral sciences, with some fascinating findings and valuable contributions. Roney et al. (2003, 2007,2010) reported a significant increase in testosterone and cortisol concentrations in men after visual exposure and brief social interactions with women. Lopez et al. (2009) conveyed that similar response occur in females to high mate-value men, much like men show in response to attractive women. Duchesne, Tessera, Dedovic, Engert & Pruessner (2012) found that both men and women demonstrated a cortisol increase when exposed to opposite sex panelists in modified version of Trier Social Stress Test (TSST). Nevertheless, we did not find any studies in research on single- sex education investigating behavior in educational settings from this perspective.

To bridge this gap, we investigated the presence of the same and opposite sex individuals in educational settings within a cognitive model of mate attraction proposed by Roney (2003). Within this model, individuals of fertile age and different sex in educational environment, will be perceived as an environmental cue or visual stimulus from potential mates (distal explanation) that prime psychological changes and elicit immediate cortisol response for mobilization of impending mating opportunities (proximal explanation). To our knowledge, this is the first attempt to look at sex from this perspective in the long endeavor of finding an explanation for why single and mixed- sex environments are qualitatively different.

Significance of the Study

Educational researchers, more times than not, tend to simplify the matter or ignore entirely the complexity of the issues involved in the investigation of educational environments, rather than devise studies that incorporate many interrelated factors. When the event explained is a piece of human behavior, no one thinks that there is only one correct causal explanation of it. Functional explanations, the sort we get from evolutionary biology, are surely consistent with the more proximal explanations of neurophysiology. Both can be correct, and both reveal part of the truth. They do not compete with but complement one another. They merely deal with different sets of causally relevant factors (Dretske, 2004).

The purpose of this pre-experimental study was to gain evidence of cortisol response of male and female participants in the presence of the same and opposite- sex individuals in social and educational settings. No studies were found that evaluate these elements in research on single-sex vs. coeducation. This investigation represents one of the few tests to date in humans of possible hormonal responses to social interactions with members of the same and opposite sex in a naturalistic setting and the first study of cortisol reactivity to same and opposite sex individuals in educational environments. As such, findings from this study may serve as proof of concept for the, heretofore, unexamined role of physiological processes in the debate over single-sex vs. coeducation.

Research Question and Hypothesis

The central question of this investigation is: does the presence of same and opposite sex counterparts elicit a hormonal response from individuals in an educational environment?

It was hypothesized that males and females (IV) grouped in both single and mixed sex environs (IV) will demonstrate significantly different cortisol responses (DV) to each environ.

Limitations and Delimitations

To test this research hypothesis, we are using a small number of individuals, as this hypothesis is novel and untested. This will avoid spending too many resources, *e.g.* participants, time and financial costs, in finding an association between elevated cortisol level and presence of the opposite sex individuals in educational environments. If an association is found in this hypothesis-generating study, a larger confirmatory study will be needed. Due to the small homogenous sample available for the study, results may not be generalizable beyond the specific population from which the sample was drawn, as racial/ethnic composition of subjects is limited only to Caucasian`s males/females. Previous endocrinological evidence suggests that African-Americans and Hispanics have flatter diurnal cortisol rhythms relative to Caucasians, both lower wakeup and higher bedtime cortisol levels (DeSantis et al., 2007); therefore it would be expected to see even more pronounced cortisol response to the presence of opposite sex individuals in African-Americans and Hispanics participants.

Organization of the Study

Chapter 1 presents the introduction, statement of the problem, research question and hypothesis, significance of the study, and limitations of the study. Chapter 2 contains the review of related literature and research related to the problem being investigated. The methodology and procedures used to gather data for the study are presented in Chapter 3. The results of analyses and findings to emerge from the study are covered in Chapter 4. Chapter 5 contains a summary of the study and findings, conclusions drawn from the findings, a discussion, and recommendations for further study.

CHAPTER 2

General Trends in Educational Research on Single-Sex Education

There are over 2000 empirical studies that are related in some way to the debate on single-sex education (Bracey, 2006). To date, several large reviews have been conducted worldwide in search of empirical evidence for or against single-sex schooling (Gill, 2004; Haag, 1998; Mael, Alonso, Gibson, Rogers, & Smith, 2005; Smithers & Robinson, 2006; Thompson & Ungerleider, 2004; Riordan et al., 2008). Ironically, for an issue that elicits strong feelings and powers highly charged debate, little conclusive evidence has been accumulated in support of either position. So, it seems, people 'know' one or the other is better but cannot prove it (Smithers & Robinson, 2006). Although suggestive that single-sex schools can benefit some students in some realms of academic and socio- emotional accomplishment, the actual research evidence is equivocal. Overall, these reviewers found a greater number of studies demonstrating more positive than negative effects of single-sex relative to coeducational schooling and nearly equal numbers of studies reported mixed or no effects of single-sex education. The few generalized conclusions that fuel these debates suggest: findings on the benefits of single-sex settings per se are ambiguous; there are some benefits for girls in single-sex settings; other factors (e.g., quality of teaching, and socio-economic backgrounds) were identified as more significant than the gender composition of the learning setting.

In support of single- sex over coeducational settings, individual studies have found that there are some benefits for girls in single-sex settings: greater positive self-concept (Lee & Bryk, 1986), less gender-stereotyping with respect to some disciplines (Lee et al. 1994), and less hampering by a "fear of success" (Winchel, Fenner & Shaver, 1974). It was also reported that these positive results were sustained upon entry into college (Lee & Marks, 1990).

Furthermore, because boys endorse cultural gender stereotypes to a greater degree than girls (Blakemore et al., 2009), classrooms that do not include males are thought to be more supportive of girls' academic achievement in counter-stereotypic domains such as math and science than classrooms that include males (Shapka & Keating, 2003). While the results of single-sex education are apparent for girls, these positive impacts are even more dramatic for African-American and Hispanic children, males and females (Riordan, 1990). Studies report that the performance of African-American and Hispanic students in single-sex schools is stronger on all tests, on average scoring almost a year higher than similar students in coeducational settings (Riordan, 1994).

Critics of single- sex education argue that the sex differences on education-relevant traits are trivially small (i.e., the distributions for males and females are highly overlapping) and thus the creation of classrooms tailored to such differences are likely to produce benefits among only a small segment of the population, if they have any impact at all (Bracey, 2006; Hyde, 2005). Other educators and researchers who oppose single-sex education argue that such schools are harmful because they reduce opportunities for cross-group contact, just as do schools segregated by race or socioeconomic status (Balkin, 2002; Campbell & Wahl, 1998). That is, these critics argue that coeducational environments are beneficial because they typically (although not always) promote tolerance and cooperation across genders, thereby reducing gender discrepancies in academic attitudes and behaviors (Eliot, 2009; Rustad & Woods, 2004). Among the studies included in the broad reviews of research concerning single-sex and co-educational schools are a number of weaknesses, both conceptual and methodological. Most significant among these is that to date there is no published study within the single- sex vs. coeducation literature that considers mechanisms by which sex is implicated in the learning environment. Having a well-grounded debate on the advantages or disadvantages of single- sex education is highly unlikely without evaluation of all factors involved in the process. These include evolved sexually dimorphic stimuli processing cognitive mechanisms of male and female students as well as their distinctive neuroendocrinological reaction to the presence of opposite-sex individuals in educational environments.

Methodologically, few of the studies reviewed were conducted using experimental designs, therefore causal claims are not widely available. The mere fact that single- sex schools are schools of choice means that from the outset, no random assignment is possible and selection effects are significantly hindering the interpretation of existing studies on single- sex education (Bracey, 2006; Campbell & Wahl, 1998; Marsh, 1989; Mael et al., 2005; Salomone, 2006). The potential presence of selection effect makes it impossible to disentangle effects of single-sex schooling per se (i.e., sex composition of schools) from effects caused by other student and school related variables (e.g., teacher quality or students' economic backgrounds). When Hayes, Pahlke and Bigler (2011) addressed this concern and conducted a study controlling for selection and peer quality effects, they found that there was no significant effect of the gender composition of schools on achievement. Distal outcomes are also exclusively used, so regardless of design, relationships between sex groupings and educational outcomes are nearly impossible to decipher, given the myriad of potentially intervening variables.

In addition, most single-sex schools that exist in the public sector in the United States are quite new, meaning that most data came from research in the public sector in other countries or from research in this country comparing public and private, usually religious, schools, or comparing single-sex religious schools with coeducational religious schools. In fact, four of the positive findings come from Lee and Bryk (1986), four from Riordan (1985, 1990, and 1994) and three from LePore and Warren (1997), all drawing on the same datasets from Catholic schools in the US. Of the other four: another involved a comparison of Catholic schools (Caspi, 1995); Carpenter and Hayden (1987) conducted a study in Queensland and Victoria; Woodward, Fergusson and Horwood (1999) compared schools in New Zealand; while the fourth was the Spielhofer et al. (2004) research in England. Moreover, Bracey (2006) noticed that because of little time available for research on single- sex schooling in the U.S., these studies would be subject to Hawthorne or John Henry effects. A Hawthorne effect is the effect of novelty—people often behave differently at the beginning of an innovation or experiment than they do later on. A John Henry effects occur when people in a group perceive that they are expected to do less well than people in another group and work harder to offset the expected deficit.

There is no doubt that research comparing the relative merits of single-sex and co-education has not yielded definitive answers. “Perhaps researchers on single-sex education have been asking the wrong questions. As the anecdotal evidence on single-sex classes demonstrate, the focus on objectively measurable short-term outcomes may simply divert attention from the real question of short-term behavioral and attitudinal changes that ultimately produce long-term effects and greater control over one’s life plan.” (Salomone, 1999)

To bridge this gap in knowledge, we propose to investigate the presence of opposite sex individuals in educational settings within a cognitive model of mate attraction offered by Roney (2003). Within this model, individuals of fertile age and different sex in educational environments will be perceived as an environmental cue or visual stimulus from potential mates that prime psychological changes and elicit physiological responses underlying courtship behavior. To our knowledge, this is the first attempt to look at sex from this perspective in the long endeavor of finding an explanation for why single and mixed- sex environments are qualitatively different.

Sex as Research Variable

As a biological category that encapsulates the anatomical, physiological, genetic, and hormonal variation existing in a species, sex determines differences in body shape and size, metabolism, hormonal and biochemical profiles, fat and muscle distribution, organ function, and brain structure (Clow, Pederson, Haworth-Brockman, & Bernier, 2009). Vast biological differences between males and females has led to the realization that "every cell is sexed", affirming the importance of including sex variables in all types of research. It is self-evident that researchers investigating the educational potential of single- sex versus coeducation identify sex as a significant variable. The integrity of the empirical foundation upon which such consideration is built is less so. Here, consideration of modern evolutionary theories is relevant.

Sexual Selection and Mate Choice

One of the primary arguments used to defend single-sex schools is that single-sex environmentsprovide relief from the distraction of sexual pressures (Caplice, 1994).
The fear of sexual distraction has been used as a reason to separate the sexes for centuries. (Balkin, 2002) asserts that "women were often seen as a cause of men's lack of success, so that it was necessary to separate them in order to ensure the men thrived". Religious schools have also used the sexual distraction logic to separate students by sex; in these cases sexual purity was seen as a sign of moral virtue. Modern concerns over sexual distraction now come from the sides of both sexes with a large emphasis placed on the effort to lower teenage pregnancy rates, the assumption being that if teenage girls are not in school with teenage boys, they are less likely to

be impregnated by these boys. Regardless of rationale, sexual pressure is recognized by many as a significant force and, if viewed from evolutionary perspective, is perfectly logical.

In humans, as in many other sexually reproducing species, individuals live under constant sexual selection pressure and frequently compete for the reproductive access to the opposite-sex partners (Buss, 1988; Walters & Crawford, 1994). Indeed, intrasexual competition has been a central driving force behind human evolution (Buss, 2007; Darwin, 1851, 1871) with sexual selection often stronger than natural selection, as it frequently drives trait values beyond their naturally selected optima. Sexual selection has long been overlooked in the human sciences, partially because evolutionary biologists themselves were skeptical about Darwin's most innovative theory until quite recently, and partially because various ideological biases kept sex marginalized as a topic too messy, too mystical, too embarrassing, and too arousing for scientific analysis. We have to face the possibility that if human evolution was a film, it would be X-rated (Miller, 1998). Despite the fact that Darwin`s The Descent of Man, and Selection in Relation to Sex was first published in 1871, virtually all of 20th century psychology, anthropology, paleontology, primatology, and cognitive science, as well as the social sciences and humanities, developed without recognizing that sexual selection could have played any important role in the evolution of the human body, the human mind, human behavior, or human culture. Since biologists have embraced sexual selection, we must face the possibility that most current theories of human behavior and culture are inadequate, because they may have vastly under estimated the role of sexual competition, courtship, and mate choice in human affairs (Miller, 1998).

In relation to single- sex education, for instance: although relief from sexual pressure is used as one of the strongest arguments to advocate single-sex programs, explanation of observed behavioral differences in single vs. mixed- sex environments from the perspective of sexual selection theory has not yet surfaced. While strong belief exists that there is a difference between single and mixed-sex environments, and explanations for this distinction is missing, perhaps sexual selection theory can be a starting point in the surge for evidence of how sex is distally implicated in a learning environment. Today, although natural selection theory serves as the conceptual and rhetorical foundation guided by evolutionary psychology research (see Tooby & Cosmides, 1990, 1992), sexual selection theory seems to lead more actual day-to-day investigations (see Buss, 1994; Ridley, 1993; Wright, 1994).

Humans, like all other sexual creatures, are subject to instinctive sexual desire triggered by appropriate criteria. Although evolution of the human body and mind has resulted in an incredibly complex psychophysiology that set human approach to reproduction apart from other animals, basic sexually dimorphic mechanisms as described by Darwin (Darwin, 1851, 1871) are the same: males compete for breeding rights and females select the best available male. Darwin's original definition of sexual selection, which emerged in The Origin of Species, appears to emphasize male–male combat [i.e., "a struggle between the males for possession of the females" (Darwin, 1851)], but even then he was clearly aware of the female`s choice. Thus, Darwin identified the two main categories of sexual selection that persist to this day: males' intrasexual competition and females' mate choice. As study of sexual selection entered its modern era during the latter half of the Twentieth century, and scientists identified the evolution of female choice as a legitimate topic, the search for mechanism of mate choice had begun (O'Donald, 1962, 1980; Zahavi ,1975; Williams ,1966; Lande , 1981; Kirkpatrick, 1982) . Once biologists started taking the possibility of female choice seriously, evidence for its existence and significance came quickly and ubiquitously (see Andersson, 1994; Cronin, 1993).

Cognitive Mechanism of Mate Choice

Mate choice is the behavioral outcome of mate preferences. These preferences are usually psychological mechanisms implemented as complex neural circuits, and constructed through the interaction of many genes and environmental conditions, which bias mating in favor of individuals with certain perceivable traits. Mate choice is simply the best eugenics and genetic screening that female animals are capable of carrying out under field conditions, with no equipment but their senses and their brains (Miller, 1998). This view implies that animals capable of perception of objects in the environment and differential aesthetic responsiveness to these objects, including potential mates (Sefcek, Brumbach, Vasquez, Miller, 2006).

Emphasizing the importance of environmental cues that might prime mating relationships, Roney (2003) proposed a model positing that (a) sensory stimuli from women can act as input cues that (b) prime a psychological orientation directed toward mate attraction that (c) facilitates the behavioral expression of courtship tactics. Social cognition research supports this model and demonstrates that the capability of objects in the environment automatically prime psychological constructs (a-b) the activation of which increases the probability of behaving in ways consistent with the content of those constructs (b-c) (Chartrand & Bargh, 1999). In two studies Roney (2003) tested a visual exposure hypothesis within this model. The logic of the visual exposure hypothesis, derived from well documented in many nonhuman species males` courtship displays in response to the sight of females (see Andersson, 1994; Jameson, 1988), bolster the fact that the mere physical presence of conspecific females is often a powerful input cue for males of vertebrate species. Roney (2003) concluded that it is logical to hypothesize that visual perception of women can prime the psychological representations that underlie mate attraction behaviors in men. As indicators of this behavior Roney (2003) used those behaviors associated with the control of material resources, as resource possession plays an important role in male mate attraction (Trivers, 1985; Kenrick, Neuberg, Zierk, & Krones, 1994; Gutierres, Kenrick, & Partch, 1999).

The first experiment tested whether the mere physical presence of members of the opposite sex could prime psychological changes associated with mate attraction. Participants in this study (10th and 12th grade high school students of both sexes) were randomly assign to testing rooms with different gender composition (same-sex vs. mixed-sex) and filled out the survey booklets without any reference being made to either the gender composition of the testing room or the identity of the target surveys. The assumption was that male students in the mixed-sex condition would perceive some of the female students as potential mates. The second experiment was an attempt to replicate and extend the results of the first study while actively manipulating the likelihood that persons to whom participants were exposed would be considered potential mates. In the second experiment, participants were University of Chicago male students exposed to magazine advertisements containing photos of either young women who were rated highly attractive or older women who were rated less attractive. Participants in the younger models' condition then rated the effectiveness of the set of ads containing the younger models, and participants in the older models condition rated the set of ads containing the older models. Results of these two experiments demonstrated that visual exposure to young women caused dramatic changes in the attitudes, mood states, and personality trait descriptions of young men. Across two very different participant populations, young men who were exposed to young women reported far more favorable attitudes toward material wealth than did men exposed to either older men or older women. Visual exposure to young women was also associated with greater feelings of ambition and aggressiveness, as well as self-descriptions indicative of high surgency/extraversion. These effects all occurred without any evidence that participants were aware of the influence of the experimental manipulations. Null results for female students in Study 1 was reported and was attributed to the sex differences in responsiveness to mere visual exposure to potential mates. The effects of visual exposure observed in males were largely restricted to items related to constructs that play important roles in female mate choice and male courtship tactics (e.g. wealth, ambition, and social status) that allowed for the suggestion that men may store a representation of female mate preferences in memory and when primed, causes the activation of a psychological orientation directed toward mate attraction. These two studies provided the first evidence (beyond self-reports of courtship tactics) for a cognitive model of mate attraction where visual stimuli from potential mates act as environmental cues capable of priming psychological changes that underlie courtship behavior.

Hormones and Behavior

Exploration of how biology and the environment interact to produce behavior is especially relevant to the single vs. mixed- sex debate and central to the illumination of precise proximate mechanism of how sex is potentially implicated in learning environment.

If one's observable behavior is an outcome produced by complex neural circuits in the brain as it processes environmental cues and elicits production of hormones that enable the mind and body to respond appropriately to the impending stimuli, then the only approach to understand behavioral responses is to understand neurophysiological circuits and the neurochemical systems that control these responses. Hormones affect behavior by influencing one or more of three "components" of behaving animals—input mechanisms (such as sensory or perceptual processes), the central processing mechanisms of the nervous system (either directly, or by affecting its development or structure), and output mechanisms (such as effectors or peripheral structures). At the same time, one's own behavior, the behavior of others, and other environmental influences can all potentially alter the functioning of the endocrine system, resulting in pervasive changes in behavior. In some cases a behavior under study and its hormonal correlates are mutually reinforcing or bidirectional: for example, higher testosterone (T) levels may lead to increased competitive encounters, which in turn may lead to increased T, which may support further competitive interactions, and so on and so on. This approach is statistically more complex to examine and interpret, but arguably more interesting and valuable because of the potential to develop a more comprehensive account of the relationship between hormones and behavior (van Anders & Watson, 2006). The areas that received enough empirical attention while investigating endocrine changes driven by environmental influences are areas that have primacy to evolution as these changes are thought to be functional because they increase survival and reproduction.

It is well documented that in most vertebrate species, males possess neuroendocrine mechanisms that regulate species-typical behavioral responses to cues from potential mates. Visual, auditory, chemosensory, or tactile cues from conspecific females are known to trigger species-specific male courtship and copulatory behaviors (Andersson, 1994). These relationships between input cues and output behaviors are mediated by phylogenetically conserved structures within a limbic–hypothalamic circuit, such as the medial preoptic area (e.g., Sipos & Nyby, 1996; for reviews, see Meisel & Sachs, 1994; Paredes & Baum, 1997). Since the hypothalamus regulates the release of sex steroids in vertebrates (e.g., Pfaff, 1981), the brain pathways that regulate responses to cues from females provide a mechanism whereby social stimuli could alter levels of sex hormones. As such, many vertebrate males exhibit a ‘‘mating response’’ to cues from potential mates: a reactive increase in sex hormone levels accompanied by species specific courtship or sexual behaviors (Roney et al., 2003). Few researchers have investigated whether a similar mating response occurs in men. Roney et.al, 2003 reported a significant increase in testosterone after brief social interactions with women. The results of this study were generally consistent with the hypothesis that human males may exhibit behavioral and endocrine responses associated with courtship behavior that are triggered by the mere presence of opposite sex and similar to that observed in males of many nonhuman vertebrate species.

Originally, changes in steroid androgen testosterone were related to reproductive behavior. As this line of research extended to other groups of vertebrates, including mammals, it evolved and a new line of research emerged that investigates the mediating effect of another steroid – cortisol - on behavior in mating and other social contexts.

Cortisol as Biomarker for Current Study

Of particular relevance to behavior are steroid hormones because they have a dualistic effect on the ontogenetic trajectory of an individual. First, they are important regulators of somatic and neural development, sexual differentiation, and early origins of adult phenotype. Second, released from endocrine glands in response to numerous environmental stimuli, they regulate both physiology and a wide variety of behaviors, including sexual behavior.

Cortisol is the most potent glucocorticoid secreted by the adrenal cortex in response to activation of the hypothalamic-pituitary-adrenal (HPA) axis, also known as the limbic-hypothalamic-pituitary-adrenal axis (LHPA) and, occasionally, as the hypothalamic-pituitary-adrenal-gonadotropic axis. Used terminology illuminates functionality of this major part of the neuroendocrine system that controls reactions to variety of endogenous and exogenous stimuli and regulates many bodily processes. The mediative power of HPA axis was mentioned earlier in the discussion of input cues from potential mates and output behaviors since the hypothalamus regulates the release of sex steroids in vertebrates (e.g., Sipos & Nyby, 1996; for reviews, see Meisel & Sachs, 1994; Paredes & Baum, 1997; Pfaff, 1981).

Individuals are constantly challenged by intrinsic or extrinsic adverse forces referred to as stressors. When challenged by a stressor, one's stress response system is activated. The stress response system enables individuals to adapt to the stressor: attention is enhanced and the brain focuses on the specific challenge or threat. Metabolism, cardiac output and respiration accelerate and blood flow is redirected to the aroused brain, heart and muscles (Tsigos & Chrousos, 2006). Two main biological systems coordinate the adaptive response of the individual to the stressor - the autonomic nervous system and the hypothalamic-pituitary-adrenal (HPA) axis, with HPA axis been a central part of this effort. The HPA consists of the hypothalamus, which secretes hormone corticotrophin-releasing factor (CRF) in humans in response to most any type of stress, physical or psychological. CRF then stimulates the secretion of adrenocorticotropic hormone (ACTH) in the pituitary glands (the second component of the HPA axis). ACTH acts on the adrenal glands (the third component of the HPA axis), which produce the glucocorticoid cortisol. Cortisol acts back on the hypothalamus and the pituitary glands to suppress the production of CRF and ACTH in a negative feedback cycle, modulating the stress response and helping the body to return to a stable state, referred to as homeostasis, a crucial component of the cycle. However, the systems that protect an individual in the short run cause damage when activated for long periods of time. Over the long term, the normal feedback system may break down and the body has trouble returning to homeostasis either due to an excessive number of stressful events leading to over-exposure to stress hormones or the body's failure to manage the hormonal stress response system (McEwen, 2000).

In the human literature, cortisol reactivity has generally been treated as a marker of negatively valenced reactions to eliciting events (for a review, see Dickerson & Kemeny, 2004). Roney et al., 2007 proposed that cortisol reactivity may also function to mobilize energy resources to help an organism to respond to positively valenced social opportunities such as the presence of possible mates in order to facilitate courtship efforts. This is a potentially novel interpretation of socially mediated cortisol reactivity, but in principle there is no reason to think that cortisol energy mobilization mechanism would not also be functional in responding to positive events. In the Roney et al., 2010 study of cortisol response in males to the presence of females, cortisol increases were associated with positive mood states and thus do not appear to be 'stress responses' in the classic sense. This argues against the common tendency in the human psychology literature to infer from cortisol increases that stimuli have been perceived as aversive or threatening (see Dickerson & Kemeny 2004), and suggests instead a broader conceptualization in which cortisol directs energetic resources towards responding to both appetitive and aversive challenges (for similar arguments, see Flinn 2006).

Recently research has addressed whether humans exhibit reactive cortisol response to various exogenous stimuli, including opposite- sex interactions. Older findings from social psychology link opposite- sex interactions to increased anxiety and discomfort in both men and women (Martinson & Zerface, 1970; Dodge et al., 1986; Chorney & Morris, 2008). The latest research examined interplay between opposite sex interactions and HPA axis activation. Duchesne, Tessera, Dedovic, Engert & Pruessner (2012) exposed 43 young women and 25 young men to a modified version of Trier Social Stress Test (TSST) where the participants gave a speech in front of a panel of judges, composed of either male or female panelists. Cortisol level was assessed in their saliva prior to the task (baseline) and at 20 min following the task onset. It was found that both men and women demonstrated a cortisol increase only when exposed to opposite- sex panelists. Interestingly, this effect was only observed in women in their follicular phase which ends with ovulation, consistent with our hypothesis that the presence of opposite sex individuals will stimulate release of species specific hormones associated with detection of potential mates. Findings from Lopez et al. (2009) reported that 120 young women in their study showed a significant increase in both salivary testosterone and cortisol after watching a brief video montage featuring a highly attractive man courting a young woman and no hormonal response to any of the three control stimuli such as pleasant nature documentary, an unattractive man engaging in courtship, and an attractive woman socializing with other women. These results indicate that women experience rapid neuroendocrine responses (within 30 min of exposure) to high mate-value men, much like men show in response to attractive women.

Roney et al., (2003, 2007) demonstrated hormonal responses (testosterone and cortisol) of men to social interactions with women providing strong evidence that human males exhibit an endocrine mating response similar to that seen in non- human vertebrate species. Parallels between the human and non-human responses include the rapid timescale of the effects (within 20 – 40 min of first exposure to females), the presence of both testosterone and cortisol increases, and the absence of hormonal responses after comparable exposure to other males. (Roney, 2010). The proposed explanation is that" the likely function of the limbic–hypothalamic pathway that regulates responses to potential mates is to couple the expression of courtship and sexual behaviors to the conditions in which such behaviors are most adaptive; reactive hormone increases are thus expected to facilitate the successful execution of mate-seeking and/or copulatory behaviors." (See Roney, 2009).

Most of the studies on the human endocrine response in relation to mating behavior were completed with only male subjects since evolutionary theories suggest that males tend to compete with one another for access to potential mates to a greater extent than females do (Smith, 1977; Trivers, 1972). However, there are many aspects of human mating that are different from other species. Indeed, there is evidence that human females compete over potential mates just as human males do (although perhaps not to the same extent) (Durante, Griskevicius, Hill, Perilloux, & Li, 2011; Fisher, 2004). Thus, the endocrinological responses of both men and women may be sensitive to the presence of potential, immediate reproductive opportunities (Lopez et al., 2009). Although acknowledging the possibility of hormonal reaction of men and women to the presence of opposite- sex individuals, there are reasons to expect differences in the hormone– behavior links between sexes. One reason is that women produce five to seven times less testosterone than men, a hormone that acts to develop the male brain for aggressive or dominant behavior in many species (Nelson & Chiavegatto, 2001). Another reason is that in women, the HPA axis is involved not only in cortisol, but also in testosterone production, while in men the production of these hormones is differentiated between hypothalamic-pituitary- adrenal and hypothalamic- pituitary-gonadal axis (Jones & Lopez, 2006).

Educational researchers, more times than not, have tended to simplify the matter or ignore entirely the complexity of the issues involved in investigation of educational environments, instead of devising studies that incorporate many interrelated factors. It is unlikely to have a well-grounded debate on advantages or disadvantages of single- sex education without evaluation of all factors involved in the process. These include the evolved sexually dimorphic nature of stimuli processing mechanisms of male and female students as well as their distinctive neuroendocrinological reaction to the presence of opposite- sex individuals in educational environments displayed in cognitive and behavioral outcomes. To our knowledge, there is no study devised to evaluate these elements in research on single- sex education. Therefore, this quantitative study is designed to gain initial evidence of salivary cortisol response of male and female participants to the presence of the same and opposite- sex individuals in educational settings, illuminate neurophysiological circuits and the neurochemical systems that control these responses and provide a possible biological explanation of how sex is potentially implicated in a learning environment.

Interpretation of Cortisol Profiles

Cortisol is the most potent glucocorticoid produced during activation of HPA and it has a variety of different effects in target systems throughout the organism, which can be summarized as aiming to increase the availability of energy substrates in different parts of the body, and allow for optimal adaptations to changing demands of the environment. Under basal conditions, cortisol secretion exhibits a 24-h circadian profile in which concentrations present a morning maximum in humans (the circadian peak), and slowly declining levels in the late afternoon, evening and nocturnal period (the circadian trough) (Lupien et al.,2007).

Investigation of cortisol response to various environmental stimuli possesses particular relevance to educationalists as the role of cortisol in sustaining and facilitating cognitive functions is well documented and supported by subjective reports and behavioral observations. Given their liposoluble characteristics, the glucocorticoids can easily cross the blood–brain barrier and access the brain where they bind to specific receptors. Three of the most important brain areas containing glucocorticoid receptors are the hippocampus, amygdala, and frontal lobes, which are brain structures known to be involved in learning and memory. The physiological, cognitive and behavioral effects of cortisol released in response to physical or psychological stressors appear to act in a curvilinear, or “inverted U shaped” fashion on many physiological and cognitive systems, in which moderate levels are optimal while extremely low or high concentrations each have distinct adverse behavioral or cognitive outcomes. For instance, short-term exposure to cortisol appears to help us learn and remember (Roozendaal 2000; Buchanan & Lovallo, 2001), but the situation reverses and cortisol acquires a negative impact on attention, learning and memory when we're exposed to it for long periods of time. (DeKloet, Oitzi & Joels, 1999; Vedhara et al., 2000; LePine, et al., 2004). This inverted-U shape function between circulating levels of glucocorticoids and memory performance can be explained by the presence of two glucocorticoid receptor types which are, similar in mediating glucocorticoid feedback effects, but differ greatly in terms of their affinity for glucocorticoids and differences in occupation of these receptors under different conditions and time of day (Lupien et al., 2007). At the time of cortisol peak (early AM phase in humans), there is activation of both Type I and Type II glucocorticoid receptors, while at the time of the cortisol trough (PM phase in humans), there is mainly activation of the high affinity, Type I glucocorticoid receptors. When Type I glucocorticoid receptors are saturated and there is partial occupancy of Type II receptors, there is maximization of memory, while when both Type I and Type II glucocorticoid receptors are not occupied (left side of the inverted-U shape function) or are saturated (right side of the inverted-U shape function), there is an impairment in memory performance (De Kloet et al., 1999). The body of literature shows that long-term potentiation (LTP), a proposed neurobiological substrate of memory formation, is optimal when glucocorticoid levels are mildly elevated, i.e., when the ratio of Type I/Type II occupation is high (see Diamond, Bennett, Fleshner, & Rose, 1992). In contrast, significant decreases in LTP are observed after adrenalectomy (surgical removal of adrenal glands), when Type I occupancy is very low (Dubrovsky, Liquornik, Noble, & Gijsbers, 1987; Filipini, Gijsbers, Birmingham, Kraulis & Dubrovsky, 1991), or after exogenous administration of synthetic glucocorticoids (Bennett, Diamond, Fleshner, & Rose, 1991; Pavlides, Watanabe, & McEwen, 1993) when occupancy is very high.

The circadian rhythm of cortisol can influence the effects of cortisol elevation on memory performance. Although elevated cortisol concentrations resulting from glucocorticoid administration or stress are typically associated with declarative memory deficits (Newcomer et al., 1994,1999; Kirschbaum et al., 1996), glucocorticoid administration during the afternoon, when cortisol concentrations are substantially lower than morning concentrations, enhanced declarative memory performance (Lupien et al., 2002).

Research showing the effects of exogenous administration of glucocorticoids on cognitive function is supplemented by studies that have assessed the effects of an endogenous increase of glucocorticoids in response to psychological stress. Given that glucocorticoids are natural substances that are secreted in the face of a challenge, a stress response has large inter-individual variations (Hellhammer, Buchtal, Gutberlet, & Kirschbaum, 1997; Kirschbaum, Kudielka, Gaab, Schommer, & Hellhammer, 1999; Kudielka, Buske-Kirschbaum, Hellhammer, & Kirschbaum, 2004; Kudielka, Schommer, Hellhammer, & Kirschbaum, 2004; Lupien et al., 1997; Pruessner, Hellhammer, Pruessner, & Lupien, 2003; Rohleder, Wolf, & Kirschbaum, 2003) and depend on the nature of the stressor. Response to absolute stressors (a real threat, such as induced by an earthquake in a town) will lead to a significant physiological stress response in every person facing this threat and is adaptive in nature. However, response to relative (an implied threat) induced by the interpretation of a situation and highly dependent on the individual perception, will be observed only in a certain proportion of individuals and may be mild or pronounced

(Lupien et al., 2006). It was determined that although endogenous increase of glucocorticoids provoked by exposure to environmental and/or psychosocial stressor will occur, stress-related elevations in glucocorticoids can have different cognitive effects on subsequent memory for material unrelated to the stressor. In humans, when a laboratory stressor (e.g., a public speaking task or a public mental arithmetic task) is administered before learning or retrieval, high glucocorticoid levels following these stressors are associated with memory impairments for material unrelated to the stressor such as neutral words lists (see Jelici, Geraerts, Merckelbach, & Guerrieri, 2004; Lupien, Buss, Schramek, Maheu, & Pruessner, 2005; Lupien, Fiocco, Wan, Maheu, Lord, Schramek,et al., 2005; Sauro et al., 2003; Takahashi et al., 2004; Domes, Heinrichs, Reichwald, & Hautzinger, 2002; Wolf, Schommer, Hellhammer, Reischies, & Kirschbaum, 2002). Recently, studies measuring the influence of stress on memory for emotional material unrelated to the stressor reported more heterogeneous findings. Thus, when a laboratory stressor was presented before learning or retrieval of emotional and neutral information unrelated to the stressor, high glucocorticoid levels following stress were associated with memory impairments for emotional information (whether positive or negative), while they had no influence on memory for neutral material (Abercrombie, Speck, & Monticelli, 2006; Domes, Heinrichs, Rimmele, Reichwald, & Hautzinger, 2004; Kuhlmann, Piel, & Wolf, 2005; Maheu, Collicutt, Kornik, Moszkowski, & Lupien, 2005; Elzinga, Bakker, & Bremner, 2005). However, two other studies showed that stress administered before (Jelici et al., 2004) or after (Cahill et al., 2003) learning enhanced memory for emotional material, while it had no impact (Cahill et al., 2003), impaired (Buchanan, Tranel, & Adolphs, 2006) or increased (Andreano & Cahill, 2006) subsequent memory for neutral information (Jelici et al., 2004).

Altogether, these results show that stress-related elevations in glucocorticoids can have different effects on subsequent memory for material unrelated to the stressor. The effects of emotionally arousing and/or stressful events on declarative memory vary according to the nature of the to-be-remembered material, with elevated levels of glucocorticoids enhancing memory for the emotionally arousing event itself but leading, more often than not, to poor memory for material unrelated to the source of stress/emotional arousal.

It was found that the time of day (morning vs. afternoon) is an important factor influencing the effects of stress-related elevations in glucocorticoids and subsequent memory. Recall that glucocorticoid receptors differ in terms of their affinity for circulating levels of glucocorticoids. Type I receptors have a 6- to 10-times higher affinity for glucocorticoids than Type II receptors. A wealth of evidence now demonstrates that activation of the Type I receptor is mandatory for successful acquisition of environmental cues necessary to encode information, whereas activation of Type II receptors is necessary for long-term memory consolidation of this information (Oitzl & de Kloet, 1992). Endogenous levels of glucocorticoids and thus, activation of Type I and Type II receptors will vary across the day, with higher endogenous levels of glucocorticoids in the AM phase compared to the PM phase. Consequently, the addition of a stressful event in the AM or PM phase, which by itself will trigger a significant increase in endogenous levels of glucocorticoids, should have a differential impact on activation of Type I and Type II receptor as a function of time of day, and consequently, on memory performance. As indicated earlier, in the AM phase, most of the Type I receptors and about half of the Type II receptors are activated, while in the PM phase, most of the Type I receptors and about a tenth of the Type II receptors are activated. If one applies a stressor in the AM phase, the endogenous increase in glucocorticoid levels that will be induced by the stressor will act by saturating Type II receptors, while the same stressor applied in the PM phase will act by activating about half of the Type II receptors. Since stress-induced elevations in glucocorticoid levels have been shown to modulate declarative memory for material unrelated to the stressor according to an inverted U-shaped function, the differential activation of Type I and Type II receptors at different times of the day thus implies that a stressor applied in the morning should impair memory function (right hand-side of the inverted U-shaped curve), while the same stressor applied in the PM phase should increase or have no impact on memory (left-hand-side or top of the inverted U-shaped curve; see Lupien, Fiocco et al., 2005).

Cortisol serves a wide range of physiological, behavioral and cognitive functions and can be elevated in a number of contexts that may or may not be “stressful”. Cortisol is produced in response to stressors, both normal (e.g. waking up, low blood sugar) and abnormal (e.g. emotional upset, infections, injury, surgery). Colborn et al. (1991) found that stallions secreted similar amounts of cortisol whether they were restrained (distress), exercised or permitted to mate with a mare (non-threatening stimuli). This pattern of cortisol response is similar in all animals, thus measuring cortisol response is a simple way to make a reasonable judgment about whether or not an organism is experiencing a stress response (reacting to a stimulus). Therefore, it might be more accurate to describe cortisol’s effects in terms of “readiness to behave” or as part of cognitive appraisal mechanisms. (Erickson et al., 2003).

Clearly, the field of psychoneuroendocrinology, which studies the effects of hormones on human brain and behavior, contributed significantly at showing the impact of stress on human cognitive function. These findings are especially relevant to the debate on single- sex education. If the endogenous increase of cortisol is detected in response to the presence of opposite- sex individuals in educational settings and given the impact of cortisol elevation on human cognition, sex should rise to the level of a relevant environmental stimulus during the tasks under investigation for the individuals and should be considered in the single vs. mixed- sex debate.

CHAPTER 3

Method

The theoretical foundation for this study was that student behavior, in part, is distally directed by sexual selection and, in full, proximally directed through the interaction of genotypic and environmental potential. The purpose of this proof of concept study was to investigate endocrinological response to single vs. coeducational environs. In support of an evolved mechanism of mate detection, it was hypothesized that males and females (IV) would exhibit significantly greater cortisol response levels (DV) in a mixed sex environs than in a single environ.

Research Design

It is noteworthy that out of almost 2000 empirical studies related to the debate on single-sex education (Bracey, 2006), very few based their conclusions on evidence attained via experiment. As random assignment to schools is impossible, researchers are unable to separate the effects of single-sex or coeducational schooling from effects caused by other student and school related variables (e.g., teacher quality or students' economic backgrounds). This speaks to the need for experimental research conducted beyond the constraints of intact schools.

There is a paucity of research concerning the human endocrine response to the presence of opposite- sex individuals in an educational environment; hence this study was designed to obtain preliminary research data of intra-individual cortisol response to the presence of the same and opposite sex counterparts in educational settings. A pre-experimental, One Group Pretest-Posttest Design was implemented, where a pretest measurement of the outcome of interest (cortisol response) is obtained prior to administering a treatment, followed by a posttest on the same measure after treatment occurs (Salkind, 2010). Although this design enables a comparison of baseline cortisol (pretest) of each participant with their cortisol level after exposure to different environs (posttest), a lack of control or comparison group prohibits extrapolation of a causal relationship between environmental sex composition (IV) and intra-individual cortisol concentrations (DV). Nevertheless, this design is a cost effective way to explore the probable causal effect of an intervention to determine if further investigation is warranted.

Conclusions made from results of One Group Pretest-Posttest Designs are limited by threats to internal and external validity. Internal validity threats include maturation, history and testing. However, the measurement of an involuntary response (cortisol secretion) limits a “learning” curve, so while the threats can’t be ruled out as possible, they can be ruled out as probable. The maturation threat is likely to come from fatigue resulting from participation in the experiment for about sixty minutes. The history threat is unlikely as participants will be isolated from events beyond the experimental condition. A testing threat is also unlikely in that study as cortisol response is an involuntary physiological response and is not learned.

External validity is jeopardized by multiple treatment interference as the same participants will be exposed repeatedly to two treatments and an effect of the previous treatment is not usually erasable. Another drawback comes from the extent to which the results of this study can be generalized to more diverse population, as our sample comprises only of typical undergraduate university students of one race- White/ Caucasians and intra-individual cortisol profiles may vary for different age/race categories (DeSantis et al., 2007).

Participants. After receiving Institutional Review Board (IRB) and Biological Use Authorization approved by the Institutional Biosafety Committee, the pre-experiment was performed in two undergraduate Foundations of Education classes at Auburn University, Alabama. Twenty students participated in the experiment, but due to cost restrictions, saliva samples of only five males (mean age =22.2 years; range 21–23 years) and four females (mean age =20.75 years; range 20–22 years) were randomly chosen and analyzed. Participants whose saliva samples were analyzed self-identified their race/ethnicity and their responses were categorized such that eight of them were White/ Caucasians and 1 mixed (Caucasian/Asian). One male participant was married and all other participants (males and females) were single. Two females reported using contraceptives. The experiment described herein was undertaken with the understanding and written consent of each participant.

Procedure. This investigation was performed during regular participant's class time between 16:00 and 18:50 as cortisol shows only small spontaneous fluctuations in the late afternoon (Weitzman et al., 1971). Participants were instructed to refrain from smoking, physical exercise, meals, and low pH drinks for at least one hour before their class time (test session) since such activities may affect cortisol levels. Upon arrival, participants were greeted by the experimenter of the same sex and guided to the room with the same sex composition as the participant. During this time potential participants were informed about inclusion criteria and given a brief Personal History and Background Questionnaire (Appendix A) to determine his or her experimental eligibility. If potential participants could answer "Yes" to at least one question, they were excluded from this study. Endocrine system dysfunction and medication taken that can obscure cortisol level were assessed. One participant (n=1) who used hormonal supplements other than contraceptives (prednisone) was excluded from the study. Demographic information, social and medical history was completed at the same time (Appendix B) and an informed consent form was read and signed by eligible participants. An experimenter in each room gave instructions about providing saliva samples in 1.5 mL labeled microcentrifuge tubes. Participants were instructed to give saliva samples by tilting their head forward and allowing saliva to collect under the tongue. Participants then transferred the saliva to the corresponding microcenrtifuge tubes by placing the open end of the tube between the lips, and then guiding the saliva into the open tube with the tongue (*Passive drool* collection *method).* After enough saliva had been collected to fill the tube at least one half full, the tubes were sealed with the provided stoppers. After 15 minutes in the single-sex environment participants provided the first saliva sample (Treatment 1-Collection 1). Each tube was labeled to indicate the sample it contained (i.e. number assigned to each participant and time of collection). This process was repeated for each data collection point. Then, the experimenters added a cognitive task- completion of a100 piece jigsaw puzzle-to the single sex experimental conditions (Treatment 2). Participants grouped themselves and formed three groups of three individuals and two groups of two individuals in only females (including sex of the experimenter) room and two groups of three individuals in only males (including sex of the experimenter) room. Participants were not prohibited to talk while completing group task to recreate a collaborative learning environment in which learners engage in a common task and have face-to-face interactions- commonly used teaching technique in educational settings. After 10, 20 and 30 minutes participants were instructed by the same sex experimenter to provide a saliva sample (Treatment 2-Collection 2, 3, 4). Once the fourth saliva sample was collected, participants were asked to stop puzzle completion and were informed that after 15 min break they would complete the same task with opposite sex counterparts present in the room. Participants were reminded to refrain from eating and drinking during break time. As males and females participants entered the same room after a 15 min break, participants then congregated into five groups with two females and one male and one group with three females and one male. Once groups were established in the mixed sex environment participants were instructed by the male and female experimenter to provide a saliva sample (Treatment 3, Collection 5). After the fifth saliva sample was collected participants spent 30 min collaboratively working on completion of the same jigsaw puzzle as in Treatment 2. After 10, 20 and 30 minutes participants were instructed by the male and female experimenter to provide a saliva sample (Treatment 4, Collection 6, 7, 8). Upon completion of the experiment, participants were offered food and drink.

Salivary cortisol collection and analysis. Hormones were measured using saliva instead of blood sampling as this technique provides several advantages. Saliva collection is less of a biohazard, less invasive, and less likely to trigger any sort of stress response associated with blood draws and potentially less likely to interfere with sexual arousal than blood sampling (Goldey & van Anders, 2011). Salivary steroids are an appropriate and widely used measure for behavioral research because they reflect the ‘‘bioavailable’’ portion of hormone that is unbound or weakly bound to binding proteins (e.g., albumin) and available to bind with receptors (Quissell, 1993). Furthermore, salivary cortisol correlates well with serum cortisol (e.g., Lippi et al., 2009; Lo, Ng, Azmy, & Khalid, 1992). Samples were transported to the Auburn University Harrison School of Pharmacy laboratory and stored at - 40 °C until the day of analysis (Salimetrics, LLC, 2010). On the day of analysis, samples were brought to room temperature to be analyzed and centrifuged, and cortisol concentrations in saliva were determined by Salimetrics, LLC high sensitivity salivary cortisol enzyme immunoassay kit as per the manufacturer’s instructions (2010). An enzyme immunoassay kit establishes active free cortisol in saliva based on the principle of competitive binding. A microtitre plate is coated with monoclonal antibodies to cortisol and cortisol in standards and unknowns competes with cortisol linked to horseradish peroxidase for the antibody binding sites. After incubation, unbound components are washed away. Bound cortisol peroxidase is measured by the reaction of the peroxidase enzyme on the substrate tetramethylbenzidine (TMB). This reaction produces a blue color. A yellow color is formed after stopping the reaction with sulfuric acid. Optical density is read on a standard plate reader at 450 nm. The amount of cortisol peroxidase detected, as measured by the intensity of color, is inversely proportional to the amount of cortisol present in a sample.

Due to the cost restrictions, only four samples from each participant were analyzed. Samples taken 30 minutes in each Treatment were selected (Collections 1, 4, 5, 8). Samples from nine participants were run in duplicate in the same assay and an inter-assay average was calculated for each participant for each of the collection time points. Cortisol immediately secreted into the blood without being stored at any point and because of its lipophilic nature, cortisol permeate through cell membranes easily and interact with intracellular receptors (Sapolsky, 1992), triggering instant physiological and behavioral responses. Circulating cortisol concentrations rise within a few minutes of the onset of HPA axis activation, and the half-life of cortisol is about 60-90 minutes. Because cortisol responses can be observed within 10– 30 min after the first exposure to the stressor, saliva samples taken after 30 min exposure to the treatment were analyzed, as endocrinological response, if occurs, should be evident at these time points. Salivary cortisol was expressed in *μg/dL,* the sensitivity was 1 *μg/dL,* and internal and external controls were included in the assays. All samples from each participant were analyzed simultaneously in duplicate. A ready-to-use, 96-well microtitre plate pre-coated with monoclonal anti-cortisol antibodies was utilized in this analysis with ready to use cortisol standards traceable to the National Institute of Standards and Technology (NIST) containing cortisol concentrations of 3.0, 1.0, 0.333, 0.111, 0.037, and 0.012 μg/dL. Two controls representing high and low levels of salivary cortisol in saliva- like matrix were utilized for further reference points. For the necessary wash buffer, a 100 mL of a 10X phosphate buffered solution, detergent and a preservative was used. The buffer concentrate was diluted 10-fold with room temperature deionized water (100 mL of 10X wash buffer to 900 mL of deionized H2O). The Assay Diluent was comprised of 60 mL of a phosphate buffered solution containing a pH indicator and a preservative. The Cortisol Enzyme Conjugate was comprised of 50 µL of a solution of cortisol conjugated to horseradish peroxidase and was diluted prior to use with assay diluent. Tetramethylbenzidine (TMB) was used as the visualizing reagent for analysis in the plate reader. The stop solution consisted of 3M sulfuric acid.

All reagents and samples were brought to room temperature. Plate layouts were established to include all controls, standards, and samples to be run in duplicate. Each plate contained four samples from nine participants (samples taken 30 min in each condition). Standards, controls, and unknowns were measured into appropriate wells along with 25 µL of assay diluent into two wells to serve as the zero value. A 1:1600 dilution of conjugate was made by adding 15 µL of the conjugate to the 24 mL of assay diluent. The diluted conjugate was immediately mixed and 200 µL was measured into each well using a multichannel pipette.

Plates were then mixed on rotator for five minutes at 500 rpm and incubated at room temperature for an additional 55 minutes. Plates were washed four times with 1X wash buffer. After each wash, the plate was thoroughly blotted on paper towels before being turned upright. Next, 200 µL of TMB solution was added to each well with a pipette. The plates were mixed on a plate rotator for five minutes at 500 rpm and incubated in the dark at room temperature for an additional 25 minutes. Then, 50µL of stop solution was added to each well with a pipette. The plates were mixed at 500 rpm for three minute on a plate rotator and incubated in the dark at room temperature for an additional 25 minutes. The plates were then read within 10 minutes of adding stop solution in a plate reader at 450 nm.

Statistical analysis. All analyses were conducted using IBM SPSS (Version 20) with alpha set at $p < 0.05$. A 2 X 4 mixed analysis of variance ANOVA was conducted. Sex was the between-subjects variable, environs was the within-subjects variable, and cortisol response level was the criterion variable. The sphericity assumption was evaluated with the Mauchly's test and the Kolmogorov-Smirnov Test was performed to validate the assumption of normality at each time point. The assumption of homogeneity of variance was assessed by the Levene's Test. Polynomial contrasts were calculated as follow-up to the time effect.

CHAPTER 4

Results

The primary purpose of this proof of concept study was to confirm the hypothesis that participants will experience a statistically significant increase in their cortisol response in mixed-sex environs compared to single-sex environs. In order to detect significant increases in cortisol responses, a 2 X 4 mixed analysis of variance (ANOVA) was conducted with sex as the between-subjects variable and environs as the within-subjects variable. Different environs comprised: single- sex environment (Treatment 1), single-sex environment with added cognitive task (Treatment 2), mixed- sex environment (Treatment 3), and mixed- sex environment with added cognitive task (Treatment 4). The sphericity assumption, which was evaluated with the Mauchly's sphericity test, indicated there was no violation, $\chi 2$ (5, N = 9) = 2.74, p = 0.74. The Kolmogorov-Smirnov Test was performed to validate the assumption of normality at each time point. Results indicated the data were normally distributed. The Levene's Test concluded there were no violations to the homogeneity of variance assumptions.

Neither the sex effect nor the sex X treatment interaction effects achieved statistical significance. However, there was a statistically significant effect of the treatment on cortisol response with a partial $\eta 2$ of 0.40, (an extremely large effect as defined by Cohen, 1988).
An effect size is summarized in Table 1.

Table 1.

Effect Size Summary Table

Source	*SS*	*df*	*MS*	*F*	*Partial η2*
Sex	.01	1	.01	.003	< .001
Treatment	.32	3	.11	4.71	.40*
Treatment X Sex	.07	3	.02	.97	.12

** Significant at p < 0.05.*

Table 2 presents the cortisol means and standard deviations for different treatments by sex.

Table 2.

Cortisol Means and Standard Deviations for Different Treatments by Sex

	Males		Females	
	Cortisol Means (μg/dL)	SD	Cortisol Means (μg/dL)	SD
Treatment 1	.885	.189	.869	.148
Treatment 2	.917	.010	.838	.179
Treatment 3	1.120	.141	1.078	.121
Treatment 4	.950	.180	1.098	.180

Figure 1 depicts the cortisol responses for different treatments by sex.

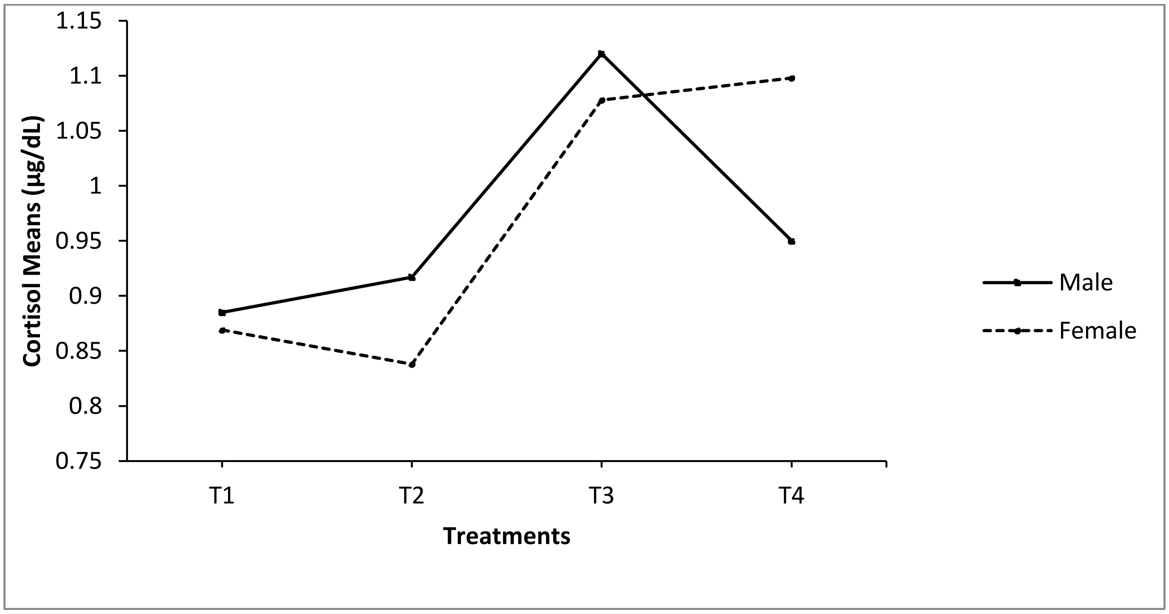

Figure 1. Graph depicts the cortisol responses for different treatments by sex.

Follow-up polynomial contrasts illustrated a significant cubic treatment effect, F (1, 8) = 7.51, p = 0.025, partial η2 = 0.48. Figure 2 presents the median, interquartile range and the smallest and the largest non-outliers in the boxplots for the treatment effect.

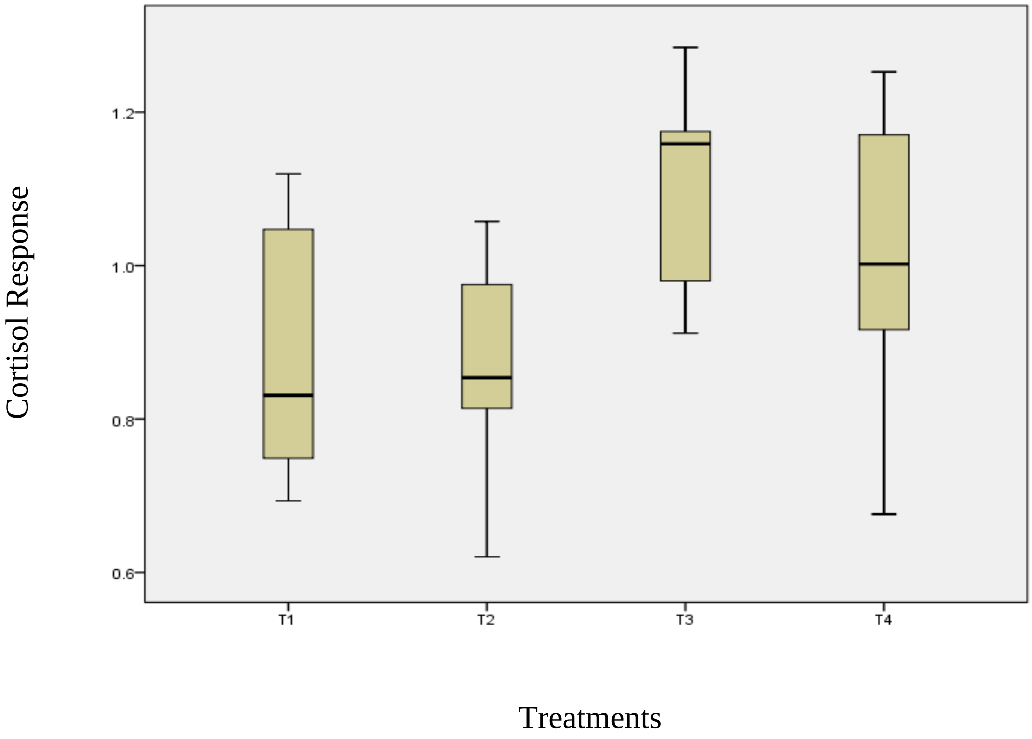

Figure 2. Boxplots showing the median, interquartile range, the smallest and the largest non-outliers in the data set of cortisol responses to different treatments.

There were no significant differences between Treatment 1 and Treatment 2. Treatment 3 demonstrated significantly greater cortisol response than either Treatment 1 or Treatment 2. However, Treatment 4 was not significantly different from Treatments 1 to 3.

Table 3 presents post hoc statistics for the relationships between treatments and cortisol response.

Table 3.

Post Hoc Statistics for Relationships between Treatments and Cortisol Response

Variable	Cortisol Means (µg/dL)	SD	95% Confidence Interval Lower	Upper	Post hoc
Treatment 1	.878	.161	.754	1.002	T1 = 2 = 4 < 3
Treatment 2	.882	.137	.777	.987	T1 = 1 = 4 < 3
Treatment 3	1.101	.126	1.004	1.198	T1< 1 < 1 < 4
Treatment 4	1.016	.186	.873	1.159	T1 = 1 = 2 = 3

The values in the Post hoc column are the relationships to the variable column

CHAPTER 5

Discussion of Findings

Consistent with the Standard Social Science Model, educational researchers, more times than not, rely on explanations steeped in cultural transmission to describe and explain student behavior. The study of single vs. mixed-sex environments is no exception. To date, the debate has been dominated by researchers who have categorically ignored the role of physiology (Kaminsky &Forbes , 2008; Mael, 2005; Tyack & Hansot, 1992). Given the interplay of biological, psychological and sociological systems, it is unlikely that the topical intransigence surrounding the debate of single vs. mixed- sex environs will be resolved unless future investigations consider these elements. The present work is an initial step in such a direction. Inspired by sexual selection theory (Darwin, 1871; Cronin, 1993; Andersson, 1994) and supported by bodies of neuroendocrinological research (Sipos & Nyby, 1996; for reviews, see Meisel & Sachs, 1994; Paredes & Baum, 1997; Pfaff, 1981; Grammer, Honda, Juette, & Schmitt, 1999; Grammer, Kruck, Juette, & Fink, 2000; Hellhammer, Hubert, & Schurmeyer, 1985; LaFerla, Anderson, & Schalch, 1978; Redoute et al., 2000; Stoleru, Ennaji, Cournot, & Spira, 1993; Roney et.al, 2003) in humans and other animals, this study considered the proximate role of cortisol response in relation to single and mixed- sex social environments with involvement of cognitive task.

Interpretation of Findings

The current study provides evidence that human cortisol response is sensitive to environmental sex composition. Participants' cortisol levels (both males and females) increased significantly in the presence of opposite- sex counterparts compared to levels in single- sex environs [$F(3, 18) = 4.71$, $p < 0.05$, $\eta 2 = 0.4$]. Interestingly, when a cognitive task was added to both the single and mixed- sex environs, cortisol levels did not significantly change. That is, cortisol response in mixed- sex environs with a cognitive task was not significantly greater than cortisol response in single- sex environs with and without a cognitive task.

These findings are the first demonstration of cortisol response to the presence of same and opposite- sex counterparts in social groups and in groups involving a cognitive task. These data complement previous findings demonstrating that the presence of opposite-sex individuals can heighten glucocorticoid levels in humans (e.g., Lopez et al., 2009; Ronay & von Hippel, 2010; Roney et al., 2003, 2007) and consistent with the hypothesis that human males may exhibit behavioral and endocrine responses associated with courtship behavior that are triggered by the mere presence of the opposite sex and similar to that observed in males of many nonhuman vertebrate species.

Compared to studies on men, relatively few studies have examined hormonal responses to the presence of opposite-sex individuals in social environments among women (for exceptions, see Roney, Lukaszewski, & Simmons, 2007; Edwards, Wetzel, & Wyner, 2006; Lopez, et al., 2009, Miller, Maner, & McNulty, 2012). The current findings support the hypothesis that, similar to men, women display endocrine reactivity to the presence of opposite- sex individuals in social environments. These findings may seem counterintuitive in light of previous research demonstrating that, across numerous species, males tend to compete with one another for access to potential mates to a relatively greater extent than do females (Smith, 1977; Trivers, 1972). Based on the literature, one might have expected sex differences in the degree to which opposite-sex individuals elicited endocrine responses reflecting competition for mates (Baker & Maner, 2008, 2009). However, there are many aspects of human mating that are different from other species. Indeed, although males of most species tend to compete over sexual access to females, there is evidence that human females compete over potential mates just as human males do (although perhaps not to the same extent) (Durante, Griskevicius, Hill, Perilloux, & Li, 2011; Fisher, 2004). Thus, the endocrinological responses of both men and women may be sensitive to the presence of potential, immediate reproductive opportunities (Lopez et al., 2009). Although the precise causal nature of observed cortisol alteration is unclear, the fact that interactions with opposite- sex counterparts produced clear effects in cortisol concentrations of both sexes suggests the importance of future research on the correlates between cortisol and presence of opposite- sex individuals in learning environs.

Limitations of the current study offer valuable directions for future research. Due to our desire for high ecological validity (i.e., wanting to examine effects in a real-world setting outside of the laboratory), we were unable to control for or manipulate various factors that might have partially caused observed cortisol responses. For example, it is possible that the greatest cortisol alteration detected after participants relocated into a mixed- sex environ, was caused by the event that happened during this transition (phone conversation, seeing someone on the hallway, etc.). By using controlled, laboratory experiments, future research may be able to address these issues and control for various factors that might have partially influenced cortisol responses. An additional limitation involves the sex ratio used in the current study; female-to-male ratio was 2:1. In settings in which there are more women than men, men may not have to compete as much for access to a potential mate (Kokko & Rankin, 2006). Thus, in such settings, men's cortisol levels would not be as high as in the setting with different female-to-male ratio. Future research would benefit from exploring cortisol responses across a broader range of sex ratios in educational environments.

It should be noted that this study focused on a small homogenous sample of four white females (mean age =20.75 years) and five white males (mean age =22.2 years). Further investigation is needed to assess the generalizability of the current findings to other populations. There are existing data (Adam, 2006; Gunnar MR, Morison SJ, Chisholm K, et al., 2001; Cohen S, Schwartz JE, Epel E, et al., 2006) that flatter diurnal cortisol rhythms are detected among African-Americans and Hispanics relative to Caucasians, driven by both lower wakeup and higher bedtime cortisol levels. It was also found (DeSantis et.al, 2007) that differences in diurnal cortisol slopes are moderated by gender among African-American adolescents, with slopes being flatter among African-American males than females. Carefully selected, representative samples will ensure that participants precisely reflect the characteristics of the larger population of interest (broader socioeconomic and racial/ethnic range than in our sample of convenience). Large sample sizes will also provide the power to identify associations that may be missed in a smaller sample due to low power.

Implications of Findings

This experiment represents one of the very few tests to date in humans of possible hormonal responses to social interactions with members of the same and opposite sex in a naturalistic setting and demonstrate the physiological effect of exposure to ecologically realistic stimuli in educational environments. It offers a new view on sex of the individual in educational research. Instead of focusing on endogenous sex differences in brain development and structure, viewing sex as an environmental stimulus and investigating how the presence of opposite-sex individuals impact student physiology and behavior can provide an explanation of how sex is potentially implicated in learning environments.

From an evolutionary perspective, glucocorticoid responses to opposite-sex individuals may function to facilitate success in intrasexual competition (e.g., enhancing ability and desires to dominate same-sex rivals; Archer, 2006), or to promote courtship displays to opposite-sex individuals (e.g., enhancing behaviors that potential mates find attractive; Ronay & von Hippel, 2010), or to do both. It was beyond the scope of this investigation to determine if cortisol response to the presence of opposite- sex individuals is an element of the evolutionary adaptation for mate choice. The hallmarks of adaptation -complexity, economy, efficiency, reliability, precision, and functionality (Williams, 1966) - are subject to empirical testing and potential falsification for any particular hypothesis about an adaptation. Because, in principle, many alternative hypotheses can account for observed cortisol deviations, a specific hypothesis is needed to conclude if observed cortisol alteration is an element of an adaptation for mate choice, a hypothesis that features an adaptation and entails a probability statement that it is highly unlikely that this feature has arisen as an incidental by-product or by chance alone. Nevertheless, findings from this study contribute to the experiential evidence that humans exhibit cortisol responses to the presence of opposite- sex individuals and might be a possible window into the design of human mating mechanisms (Roney et al., 2003; 2007). Thus far, there is little empirical evidence that such cortisol responses are a part of the mechanism of mate choice in humans. Such a hunch, however, can be useful in guiding further investigations.

For educators, though, proximate effects of cortisol elevation are more relevant, considering that glucocorticoids can easily cross the blood–brain barrier and access the brain where they bind to specific receptors in the hippocampus, amygdala, and frontal lobes. These structures are implicated in learning and memory so determining the relation between sex groupings and cortisol production is vital for educators. The role of cortisol in sustaining and facilitating cognitive functions is well documented and supported by experimental physiological data, as well as subjective reports and behavioral observations. The physiological, cognitive, and behavioral effects of cortisol appear to act in a curvilinear, or “inverted U shaped,” fashion on many physiological and cognitive systems, in which moderate levels are optimal while extremely low or high concentrations each have distinct adverse behavioral or cognitive outcomes (Erickson et al., 2003). For instance, in moderate concentrations, cortisol appears to help us learn and remember (Roozendaal 2000; Buchanan & Lovallo, 2001), but the situation reverses and cortisol has a negative impact on attention, learning, and memory at higher concentrations or when we are exposed to it for long periods of time. (DeKloet, Oitzi, Joels, 1999; Vedhara et al. 2000; LePine, et al. 2004). Elevated cortisol can block chemical reactions in the brain that are necessary for learning as it forces the brain to react first to the challenges imposed by the environment. These data are processed first, shifting our attention from cognitive processes down to the faster-acting limbic system, while more complex cerebral operations shut down.

Now, when an association is found between elevated cortisol levels and the presence of opposite- sex individuals in educational settings, these data can be used to analyze possible implications for learning, cognitive development, and pedagogy. Observed increases in cortisol concentrations in response to the presence of opposite- sex individuals in educational settings and the impact of cortisol elevation on human cognition brings sex to the level of a relevant environmental stimulus and calls for advancing a bio-psycho-social position that welcomes multi-disciplinary perspective into the single vs. mixed- sex debate. This holistic approach can also be used to analyze already documented findings in educational research on single- sex education. For instance, the research evidence indicative that single- sex schools significantly benefit minorities, especially males (Riordan, 1994), may be informed by the distinction in diurnal cortisol slopes among African-Americans and Hispanics relative to Caucasians (Adam, 2006; Gunnar MR, Morison SJ, Chisholm K, et al., 2001; Cohen S, Schwartz JE, Epel E, et al., 2006) with differences in diurnal cortisol profiles moderated by gender among African-American adolescents (DeSantis et.al, 2007).

Though the primary goal of this study was to test the mere existence of cortisol responses to same and opposite- sex individuals in educational settings, there are clearly many unanswered questions and broad opportunities for new venues of research. There are potentially interesting questions regarding individual difference variables that may predict which individuals exhibit this sort of hormonal reactivity, given that salivary cortisol responses show large intra- and inter-individual variability. It is a temptation to speculate that salivary cortisol responses to various stressors are influenced, at least in part, by personality variables (Kirschbaum et al., 1992, 1995; Pruessner et al., 1997).

Furthermore, examination of the long-term effect of cortisol elevation caused by repeated exposure to the opposite sex can provide valuable input for the single vs. mixed- sex debate. If consistently higher intra-individual cortisol levels persist in educational settings with mixed- sex composition, it can be extrapolated that the presence of opposite- sex individuals contributes to naturally high in the morning basal cortisol levels (Lupien et al., 2007) and creates an adverse effect on cognition (DeKloet, Oitzi, Joels, 1999; Vedhara et al. 2000; LePine, et al. 2004). Although basal levels are typically regained after 60-90 min, prolonged periods of exposure to a stressor can lead to a cortisol secretion that is sustained over several hours (Kudielka & Kirschbaum, 2004). If activated for long periods of time, the normal feedback system may break down, and the body has trouble returning to homeostasis due to over-exposure to cortisol and other stress hormones, which is detrimental to overall health (McEwen, 2000).

Another possible direction for future research lies in the developmental variances of cortisol reactivity. Recent developmental studies indicate that salivary cortisol responses to psychological stressors in children and adolescents show age-related changes in HPA reactivity to the same stressor (Gunnar, Talge & Herrera, 2009). Investigating at what age the presence of opposite- sex individuals starts to adversely impact students' cognition can be useful for the single vs. mixed- sex debate, as it can help to determine when sex as an exogenous element becomes a decisive grouping factor.

Many factors affect each child's learning profile and preferences. Family upbringing and socioeconomic status are among these. There is evidence that stressful pre- and postnatal life experiences potentially exert a lifelong impact on HPA axis responses to diverse psychological challenges (Huizink et al., 2004; Luecken & Lemery, 2004; Weinstock, 2008; Luecken & et al., 2006). Indeed, birth weight was inversely related to salivary cortisol responses to acute psychosocial stress in adult males and boys (Wu¨st et al., 2005; Phillips et al., 2006). Furthermore, a study on the effects of high familial adversity in early childhood on salivary cortisol stress responses to unfamiliar situations suggests a significant gene-environment interaction (Ouellet-Morin et al., 2008). These findings demonstrate that an array of psychological and social factors have an impact on the cortisol response of an individual, calling for consideration of these factors in all areas of research, including educational, that is relevant to single vs. mixed- sex education.

An interdisciplinary approach to current educational challenges that involves drawing appropriately from multiple disciplines (i.e. evolutionary biology and neuroendocrinilogy) will help to redefine problems outside standard boundaries of educational research and reach solutions based on a new understanding of complex situations. Findings that come from interdisciplinary studies can provide educational practitioners and policymakers with information about how hormonal responses impact sexually dimorphic cognitive mechanisms, making it possible to reach a scientifically informed decision about which educational environment – single- sex or coeducational - is the most optimal for learning.

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APPENDIX A

PERSONAL HISTORY & BACKGROUND QUESTIONNAIRE

If you can answer "Yes" to at least one question, you are not eligible to participate in this research study.

Are you experiencing or have been diagnosed with any of the following illnesses or conditions and /or are taking glucocorticoids drugs?

Illness or Condition

___ Hypotension

___ Fibromyalgia

___ Hypothyroidism

___ Chronic fatigue syndrome

___ Arthritis

___ Premature menopause

___ Feeling tired despite sufficient hours of sleep

___ Addison's disease

___ Cravings for salt

___ Poor immune function

___ Reliance on stimulants like caffeine

Illness or Condition

___ Fatigue

___ Insomnia

___ Abdominal weight gain

___ High blood pressure

___ Irritability, anxiety, or depression

___ Weak muscles

___Cushing's syndrome

___ High blood sugar

___ Cravings for sugars

___ Intolerance to cold

Glucocorticoids drugs (brand names are given in parentheses)**:**

- Betamethasone (Celestone)
- Budesonide (Entocort EC)
- Cortisone (Cortone)
- Dexamethasone (Decadron)
- Hydrocortisone (Cortef)
- Methylprednisolone (Medrol)
- Prednisolone (Prelone)
- Prednisone (Deltasone)
- Triamcinolone (Kenacort, Kenalog)

APPENDIX B

CODE____________

DEMOGRAPHIC INFORMATION

GENDER (circle): Male Female

BIRTH DATE: /_____/_____/_____/
Month Day Year

ETHNICITY (circle any that apply)**:**

Caucasian; American Indian/Alaskan Native; Asian; Black/African American;

Hispanic/Latino; Hawaiian/Pacific Islander; Other

☐ I confirm that I was refrained from smoking, physical exercise, meals, and low pH drinks for at least 1 hour before entering this study.

☐ I **did not** confirm that I was refrained from smoking, physical exercise, meals, and low pH drinks for at least 1 hour before entering this study.