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PUBERTAL TIMING AND COGNITVE ABILITY IN YOUNG ADULTS: TESTING HYPOTHESES ABOUT BRAIN SENSITIVITY TO SEX HORMONES.

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ABSTRACT

The purpose of this study was to examine indirectly the effects of gonadal steroids on the brain during puberty by assessing cognitive ability. Specifically, this study compared two relevant hypotheses regarding pubertal sex steroids and cognition using retrospective pubertal data in college aged participants. Waber hypothesized and found that late maturing individuals performed better and early maturing individuals performed worse at Mental Rotations regardless of sex, indicating only pubertal timing affected cognitive ability. Sisk however found that hamsters that experienced early and on time testosterone surges, mimicking early and on time puberty, showed more male sex-typical behavior, such as mounting behavior. This indicated that behavior may be influenced by pubertal timing and may differ depending on sex. This also indicated that there may be a window of time when the brain is most susceptible to the effects of pubertal hormones. Data was collected from classified as early, on-time, or late pubertal maturation Forty-one participants were classified as either early or late pubertal maturation (early males N=6, late males N=8, early females N=34, late females N=30). Findings replicated previous work on Mental Rotations but not on Verbal Fluency. There was an interaction between cognitive ability and pubertal timing on Verbal Fluency but not Mental Rotations. Results do not provide support for a role of pubertal hormones in organizing the brain to subserve the sex-typed cognitive abilities of spatial ability and verbal fluency. But, study limitations – especially limited statistical power for men – make it difficult to conclude that such effects do not exist.

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Introduction

Human behavioral sex differences have been examined in relation to genes, physiology, and socialization. For years, people have looked to sex steroids as an explanation for human behavioral sex differences (Blakemore, Berenbaum, & Liben, 2009). To date, animal research has been vital for gathering information about the effects of sex steroids' on behavior. During sensitive periods of development, it is thought that sex steroids act to influence the brain, and in turn, influence behavior (Arnold & Breedlove, 1985). Recent research has focused on the mechanisms by which sex steroids act on the brain and when in life the brain is most sensitive to their influences. The focus of this work is to examine indirectly when the brain is most susceptible to the organizational effects of gonadal hormones by looking at pubertal timing. The emphasis of this research is on cognitive ability because there have been varying results about how cognitive ability is affected by pubertal hormones.

Sex steroids, which are secreted prenatally and during puberty, act on the brain through different mechanisms and are thought to contribute to physical and behavioral sex differences (Collaer & Hines, 1995). The Hypothalamus Pituitary Gonadal (HPG) Axis secretes sex steroids which act to organize and activate brain pathways and contributes to brain development during puberty(Romeo, 2003; Romeo, Richardson & Sisk, 2002).The hypothalamus secretes GnRh which acts on the pituitary gland to secrete Luteinizing Hormone (LH) and Follicle Stimulating Hormone (FSH). LH and FSH then act on the maturing testes to secrete testosterone in boys and act on the maturing ovaries to secrete estrogen in girls (Gitlin & Biasucci, 1969). The sex steroids are responsible for determining internal reproductive systems prenatally and for secondary sex characteristics that are observable in adolescents during various stages of puberty

(Tanner, 1962; Palmert & Boepple, 2001). High levels of prenatal androgens are responsible for developing internal male reproductive systems and inhibiting internal female reproductive systems with the exception of Complete Androgen Insensitivity Syndrome (CAIS). Individuals with CAIS produces high levels of androgens but their body fails to recognize the hormone, causing an abnormal internal reproductive system and female typical external genitalia (Money, Ehrhardt & Masica, 1969). The absence of high androgen levels result in internal female reproductive systems (Abramovich, 1974; Donahoe et al., 1977). Androgen is therefore typically thought to be a major driving force in sexual differentiation of both internal and external reproductive features in humans. Postnatal sexual differentiation of behavior due to sex steroids is also seen in animal populations. Typically, testosterone is the sex steroid that has been thought to be responsible for sexual disparities in mating behaviors observable around the time of puberty (Romeo, 2003). In Syrian hamsters, the HPG axis determines the timing of post-natal testosterone secretion which subsequently influences adult behavior in both neonatal and adolescent time periods (Schulz, Molenda-Figueira & Sisk, 2009).

Research which shows the mechanisms by which the sex steroids act on the brain is ongoing, but animal research to date has been important in showing two means by which sex steroids have the possibility to influence the brain. Gonadal hormones can work by means of activational effects or organizational effects (Collaer & Hines, 1995). Organizational effects structure and arrange neural pathways (Arnold & Breedlove, 1985). Activational effects, in contrast to organizational effects, activate neural pathways already present. Phoenix et al. proposed The Organizational Hypothesis and were pioneers in showing that sex steroids, present in early stages of development, alter behavior thought to be caused by alter brain function. Phoenix et al. found that sex hormones administered prenatally had significant lasting effects in

adult guinea pigs. Female guinea pigs that received testosterone prenatal showed similar external genitalia and mating behaviors later in life to control male. In addition, both male and female guinea pigs that received prenatal testosterone showed more male-typical mating behaviors than female guinea pigs gonadectomizied and receiving testosterone treatments in adulthood (Phoenix et al., 1959). The Organizational Hypothesis therefore states that androgens can alter neural systems that mediate mating behavior (Phoenix et al., 1959; Wallen, 2009). Since 1959, much research has been devoted towards testing, challenging, and refining The Organizational Hypothesis. Specifically, research has focused on expanding the species on which The Organizational Hypothesis first applied to and has focused on including more sex differences due to sex steroids other than mating behaviors.

Behavioral research also shows that sex steroids can have organizational effects on the brain during prenatal sensitive periods in human beings. Individuals with disorders of sex development have become a model to understand the organizational effects of sex steroids. Some of the most compelling research comes from studies of individuals with Congenital Adrenal Hyperplasia (CAH). Congenital Adrenal Hyperplasia is an autosomal recessive disorder characterized by excessive prenatal androgen exposure as a result of steroid 21-hydroxylase deficiency (Speiser & White, 2003). Sex-typed activities and cognitive abilities have become highly explored areas of research in search of evidence for organizational effects due to sex steroids.

Toy preference has been studied in relation to levels of prenatal androgen exposure. Control boys and girls differ in their preference for toys in that control boys prefer “boys’ toys” while control girls prefer “girls’ toys. “Boys’ toys consist of toy vehicles and construction toys and “girls’ toys” consist of dolls and kitchen supplies (Berenbaum & Hines, 1992). Girls with

CAH spent about the same amount of time as control boys and more time then control girls playing with "boys' toys"(Berenbaum & Hines, 1992; Berenbaum & Snyder, 1995). The severity of the CAH, which correlates with degree of androgen exposure (Speiser et a1., 1992), was also correlated with degree of male typical behavior in that the more severe the disease, the more masculinized the toy preference was (Nordenstrom et al., 2002). The results of these behavioral studies may be mediated by socialization since girls with CAH may be exposed to more boys' toys earlier in life, perhaps from parents who have knowledge of their disorder, influencing girls with CAH to prefer boys' toys. Taking in to account socialization, studies of toy preference in girls with CAH produce evidence that prenatal androgens, acting on the brain, produce behavioral effects later in life.

Research on individuals with CAH also shows that prenatal androgen exposure may affect sex-typed cognitive ability, such as in spatial mental rotation tests, where males have an advantage (Collins & Kimura, 1997; Voyer, Voyer, & Bryden, 1995). Verbal tasks however, such as verbal fluency, tend to show a slight female advantage (Blakemore, Berenbaum & Liben, 2009; Peters, Reimers & Manning, 2006). Girls with CAH also showed lower verbal speed than control girls in a perceptual speed test (Hampson, Rovet & Altmann, 1998) but higher spatial ability than control females on spatial tasks such as Hidden Patterns, Card Rotations, Mental Rotations (Resnick et al., 1986), Spatial Relations (Hampson, Rovet & Altmann, 1998), and the Virtual Water Maze (Mueller et al., 2008). There appears to be a direct influence of androgens on spatial cognition because females with the most severe form of CAH (Salt-Wasting) performed most similar to males in a computerized virtual water maze (Mueller et al., 2008). There is substantial evidence that spatial ability in individuals with CAH is influenced by prenatal androgen exposure. We must however take into account that the differences seen in

cognitive ability in individuals with CAH may be affected by socialization. Twin studies show that females who have male twins show higher mental rotation ability then control females. This may be due to the fact the female twin is exposed to more masculine activities (Vuoksimaa et al., 2010). It is therefore plausible that females with CAH may be socialized more similar to males, for example exposed to more masculine activities, causing cognitive ability to appear more masculinized (Berenbaum & Beltz, 2011). A follow up sibling study however did show evidence that testosterone effects on spatial ability are less likely to be due to socialization and more likely to be due to prenatal androgen exposure. Females with the opposite sex co-twin performed better on the Mental Rotation task then females with the same sex co-twin and non-twin females who had an older brother (Heil et al., 2011).

Research involving girls with CAH, as described above, shows support that behavior observed later in life may be due to the organization effects of sex hormones that act on the brain during prenatal time periods. Recently however, it has been hypothesized that sex steroids can also act to organize the brain during other periods of development. In animal models, Arnold and Breedlove showed that brain alterations, by way of sex steroids, can also occur later in life. They hypothesized that sex steroids organize developing neural circuits at a cellular level by altering morphology, such as altering neuron number or growth (Arnold & Breedlove, 1985). Arnold and Breedlove pointed to evidence from female zebra finches: female zebra finches given estrogen prenatally then further treated with testosterone in early adulthood (> 90 days of age) showed neuronal growth later in life (Gurney, 1981). The birds were sacrificed in adulthood so although long-lasting effects in late adulthood were predicted, the results are certain. As Arnold and Breedlove suggested, since neuronal growth occurred later in life as a result of testosterone administered after prenatal time periods, organizational effects may not be limited to early

developmental. This was exciting new evidence that built upon The Organizational Hypothesis by expanding the time period when the brain may be sensitive to sex steroids.

It has also been hypothesized that puberty is another sensitive period when the brain has the potential to be influenced and organized by sex hormones (Sisk & Zehr, 2005). The two major sex steroids that act during puberty, testosterone and estrogen, may influence the brain in different ways.

Testosterone, which is a sex steroid secreted in high levels in males and lower levels in females during puberty, accounts for many changes and developments in the brain in both animals and human beings (Goldstein et al., 2001; Gibbs, 2005; Janowsky, 2006). The hippocampus, which is involved in spatial memory, cognition, and learning (Eichenbaum, 2000; Maguire et al., 2003), was shown to be altered by pubertal androgens and showed increased synaptic plasticity during puberty in hamsters (Schulz, Molenda-Figueira & Sisk, 2009). Changes in the human brain were also observed by MRI imaging during puberty. Changes in levels of circulating testosterone in boys and girls, due to puberty, were correlated with differences in the amygdala and in the hippocampus in humans (Neufang et al., 2008). Pubertal girls showed greater hippocampal volume than pubertal boys (Neufang et al., 2008). This may be the result of lower circulating testosterone in girls during puberty. Increased levels of circulating testosterone also paralleled volume increases in the hippocampus and paralleled the number of androgen receptors in the amygdala (Neufang et al., 2008).The results from this study helped to show that some sex differences in the brain may become prominent during puberty when the gap between testosterone levels in boys and girls begins to widen again. This suggests that puberty may be a developmental time period when the brain is susceptible to the organizational effects of testosterone.

Recent research in animals has shed light on the impact of estrogen as a major contributor in neuronal organization during puberty in human beings. Ovarian hormones have been shown to play a role in sexual brain differentiation in rats. Neuron number in the visual cortex of female rats, which is typically 19% less than male rats (Reid & Juraska, 1992), was significantly reduced when ovarian hormones were secreted, regardless if testosterone was administered. Ovariectomies performed on the female rats, however, prevented the visual cortex volume loss (Nuñez, Sodhi & Juraska, 2002). This indicates that the sex difference seen in the visual cortex is driven by ovarian hormones, since cell death only occurred when estrogen was present (Sisk & Zehr, 2005). In a separate study, the effects of estrogen on mating in Long Evans rats were studied by the removal of the ovaries in three stages: directly after birth, peripubertally, or in adulthood. Researchers then examined the influence of ovarian steroids on sex-typical mating behavior. Male rats, when protecting their food, typically will move in a pattern around their mid-body while females will move in a pattern around their pelvis. Female rats displayed male typical food protection behaviors when they received an ovariectomy, which resulted in the absence of ovarian hormones either directly after birth or before puberty. Female rats that had ovariectomies in adulthood however did not display male typical food protection behaviors (Field et al., 2004). This is further evidence that ovarian hormones, like androgens, may contribute to organizational changes in the brain which result in sex-typical behavior in animals. This research has led people to question whether ovarian hormones also impact human sex-typical behavior. When correcting for age, one study found that higher levels of estrogen in pubertal girls were associated with a lower overall gray matter volume (Peper et al., 2008). Clinical studies of girls with Turners syndrome, which is characterized by low levels of estrogen (Saenger, 1996), examined neuroimaging before estrogen therapy was administered. Results

show that girls with Turners syndrome have lower gray matter volume in specific areas of the brain that may correlate to certain cognitive-behavioral phenotypes when compared to control girls (Marzelli et al., 2011). Future research should follow up with studies involving pubertal girls with Turners syndrome to provide more evidence ovarian hormone to brain morphology. Little human research however has been conducted connecting ovarian hormones to brain developments during puberty because it was classically thought that testosterone drives brain sex differences (Zuloaga, 2008). The organizational effects of estrogen during puberty may become more clear when research is conducted comparing the effects of ovarian hormones to timing of puberty (Schulz, Molenda-Figueira & Sisk, 2009).

Timing of puberty can help address pubertal organizational effects by providing insight into when the human brain is most sensitive to gonadal hormones. Human research has primarily focused on studying behavior during and after puberty in order to examine organizational effects of sex hormones, as opposed to manipulating hormones as seen in animal studies. Psychopathology in relation to timing of puberty has been studied at length. It is important to point out that behavioral changes in relation to pubertal timing may be driven by social factors, not necessarily active hormones during puberty. Eating disordered behavior and depressive symptoms are commonly studied in regards to pubertal timing in which early maturers show greater disordered eating behaviors and elevated depression rate. It had been shown that individuals with early maturation are at greater risk for negative outcomes such as substance abuse, criminal behavior and sexual activity (Copeland et al., 2010). Many negative outcomes may be a result of socialization with peers. For example, early maturing girls who have developed secondary sex characteristics before other girls their age may associate with older kids and as a result having them to become active in older activities not suitable for girls their age.

Studies therefore first set out to examine if pubertal hormones, rather than socialization, play a role in psychopathological changes. Levels of circulating pubertal hormones, indicating possible activational effects, were related to disordering eating. In one such study, twins with high levels of estradiol showed the greatest disordered eating behaviors while twins with low circulating estradiol showed little disordered eating behaviors. This suggests that estradiol secretion during puberty may moderate genetic factors that influence disordered eating behaviors (Klump et al., 2010). This was not a longitudinal study however and cannot conclude that the disordered eating behaviors, which may be moderated by differing levels of estrogen, last into adulthood indicating organizational effects. Early pubertal timing has also been associated with increased depression and anxiety (Mendle et al., 2010). Although negative psychological outcomes are seen commonly seen in adolescents who encounter abnormal pubertal timing in comparison to peers, evidence to date is more supportive that psychological effects are limited to adolescence and may be influenced by social factors and or may indicate activational effects rather than organizational. This may be the case since sex steroids most likely activate neuronal circuits which mediate psychological outcomes during puberty as opposed to permanently changing brain pathways. Evidence to date is inconsistent in finding long term psychological effects (Copeland et al., 2010; Reardon, Leen-Feldner & Hayward,2009). Negative outcomes for early maturers seem to disappear as other peers go through puberty, suggesting that socialization may have more of an effect on psychopathology during puberty then do pubertal hormones (Natsuaki, Biehl & Ge, 2009).

In addition to psychological effects due to timing of puberty, some research has been conducted in relation to cognition during puberty, which showed varying results. In 1976, Waber was a pioneer in research relating maturation rate to cognition. She proposed and found that

individuals with early maturation perform better at verbal tasks than spatial tasks and late maturing individuals perform better at spatial tasks than verbal tasks regardless of sex. The mechanism for sex and maturation differences was first proposed to be brain lateralization (Waber, 1976). Other researchers found varying results when replicating Waber's work. Some found that there were no significant correlations between spatial ability and maturation rate (Strauss & Kinsbourne, 1981; Waber et al., 1985; Newcombe, Dubas & Baenninger, 1989). Another study showed there was both a maturation rate and sex component to spatial ability, which adds upon Waber's work which only found a maturation rate component to spatial ability. Retrospective data of timing of puberty, indicated by timing in relation to peers, as well as sex was found to be correlated to spatial ability where late maturers showed higher spatial ability than early maturers and males showed higher spatial ability then females. This study however found no correlation between age of menarche, a common predictor for timing of puberty, and spatial ability (Sanders & Soares, 1986).

Researchers have also tried to connect cognition with pubertal hormones via indirect hormonal assays. Petersen measured hormonal influence on cognitive abilities by measuring secondary sex characteristics. She measured level of androgyny by physical development, body shape and pubic hair ratio during three different periods of adolescence. She also measured fluency and cognitive abilities during those periods. Petersen found that less masculine males scored lower on fluency tasks and higher on spatial tasks, while more masculine males scored the reverse (Petersen, 1976). Replicating studies however found differing results from Petersen and little evidence for lasting effects into adulthood (Berenbaum & Resnick, 1982). Cognitive abilities in relation to androgyny may have also been mediated by social influences. If a more

androgynous male for example was socialized more like female, cognitive differences may be attributed to socialization not hormonal influences.

Few longitudinal studies have followed up on this topic but some suggest that there still is a link between puberty and spatial ability. A study related testosterone and estrogen samples taken through saliva to longitudinal cognitive ability. Boys and girls during puberty were given cognitive tasks and had their hormone levels tested. Follow up cognitive tasks were done every year from ages 14-17. Greater long-term spatial ability was found in boys with initial higher testosterone levels while no relation to cognitive ability was found in girls (Hassler & Nieschlag, 1991). A follow up study, conducted a year later, however to found no relationship between hormone samples taken through blood analysis and cognitive ability (Hassler, 1992). Davison and Susman found a positive linear relation between spatial ability tested at three different stages and testosterone in boys (Davison & Susman, 2001). Some of the most compelling work linking puberty to cognition comes from clinical samples. A study of individuals with Isolated Hypogonadotropic Hypogonadism (IHH) found impaired spatial ability in affected males in comparison to men with normal steroidal function (Hier & Crowley, 1982). Males with IHH have nearly normal masculinization due to androgens prenatally but impaired steroid production at puberty (Collaer & Hines, 1995). It is therefore speculated that androgen plays a role in the development of cognitive ability during childhood or puberty (Hier & Crowley, 1982). In addition, research has been conducted in order to replicate Waber's work using a clinical sample. Girls with central precocious puberty, in which the onset of puberty occurs extremely early, were found to have lower spatial functioning than controls. This difference in timing of estrogen release during puberty between girls with central precocious puberty and control girls may be a factor in the difference in spatial ability. This indicates that early exposure to sex steroids, due to

the abnormally early onset of puberty, may affect cognition in females (Ehrhardt, & Meyer-Bahlburg, 1986).

Research involving girls with central precocious puberty sparked an interest in the fact that estrogen, not just androgens, may also influence cognitive ability during puberty. Patients with Turners syndrome, showed lower scores in verbal ability in comparison to the normal percentile of girls (Money and Alexander, 1965). In another study of Turners Syndrome, girls ages 5-12 were given either estrogen treatments or a placebo, and then given cognitive and motor tasks. Findings show that girls aged 12 years old with estrogen treatments showed increased speed and ability on nonverbal processing tasks and motor tasks when compared with girls aged 12 years old given the placebo (Ross et al., 1988). These findings show evidence that pubertal estrogen may have an effect on cognitive ability however there are few longitudinal studies to show support that the effects are lasting. Studies that directly tested hormonal samples taken at puberty in normal populations however found little support that lower estrogen levels are correlated with great spatial ability (Davison & Susman, 2001). Due to varying results in research involving the impact of pubertal hormones on cognitive ability, research is still ongoing.

The mechanism by which puberty is another sensitive period for brain development has been a focus of recent studies. Nonhuman research is important in explaining the model of sensitivity. Originally, Sisk provided a model which explained that perinatal and pubertal periods were two separate times of brain sensitivity (Sisk, Schulz & Zehr, 2003). She modified her model based on evidence suggesting that there may be a continuum of brain sensitivity (Schulz, Molenda-Figueira & Sisk, 2009). In the newest model, Sisk suggests that the brain sensitivity occurs prenatally during a testosterone surge, there is then a period of unknown testosterone dependent organization until puberty. At puberty, when testosterone peaks, brain

sensitivity again occurs then again declines over the course of puberty. During puberty, steroid sex hormones peak and may refine original neuronal circuits and organize new neural circuits (Sisk & Zehr, 2005).

Research involving rodents and pigs support Sisk's theory of declining brain sensitivity during puberty. The pubertal time period acts as a window of sensitivity in which sex steroid hormones may be an integral part in organizing and activating brain receptors responsible for masculinizing or feminizing behavior (Wallen & Baum, 2002). In one study, two categories of Syrian hamsters, those given testosterone hormones during puberty and those in which testosterone treatment was absent during puberty, were given 17 days of testosterone treatment in adulthood. A disparity in mating behaviors was seen between the two groups of hamsters. Those given testosterone treatments exhibited more male like mating behaviors compared to hamsters where testosterone was absent during puberty. Hamsters that did not receive testosterone treatments during puberty exhibited significantly less male like mating behavior even when testosterone was administered in adulthood in attempt to reduce the disparity. This establishes evidence that the sex hormone testosterone secreted during puberty produces lasting behavioral effects into adulthood. In addition, given the fact that testosterone treatment in adulthood did not reduce mating disparities between the two groups of hamsters, the window of sensitivity during puberty may be dependent on the stage of puberty and may eventually close (Sisk & Zehr, 2005).

Most recent animal research has focused on the timing of pubertal maturation in order to establish if and when during puberty the brain declines in sensitivity. In one study, male hamsters were castrated and given either testosterone implants or a placebo before, during, or after normal pubertal development. Hamsters with early and on time puberty, but not late pubertal timing, were found to have normal mating behaviors. Early maturers were also found to

have the most effective mating behaviors. This supports the fact that brain sensitivity to sex hormones may peak during the initial stages of puberty and then decline during subsequent stages of puberty (Schulz, Molenda-Figueira & Sisk, 2009). Research about brain sensitivity during different stages of puberty in humans is the next step toward expanding the declining brain sensitivity theory.

Evidence to date, including non-human and human studies, show that sex hormones released prenatally have organizational effects on the brain that influence sex-typical behavior. However, as The Organization Hypothesis continues to be refined to include puberty as a sensitive period of development, research must now concentrate on how the declining sensitivity model may help to explain at what points during puberty the brain is sensitive to sex hormones. Recent animal research of pubertal hormonal effects on the brain during a period of what is thought to be a declining window of sensitivity now needs to be expanded to include research from human beings (Berenbaum & Beltz, 2011). The purpose of this study therefore is to test Sisk's ideas of declining sensitivity during puberty on cognitive abilities by using retrospective pubertal data in humans. Studying timing of puberty, which is indicative of when sex hormones are released and have the potential to act on the brain, is a non-invasive method to indirectly study the effects of sex hormones on the brain during puberty. Cognitive ability, which as described above may be influenced by the surge gonadal hormones prenatally, may also be influenced during puberty when the gonadal hormones secretion peaks (Reiter & Grumbach, 1982). Cognitive ability is a good topic to relate to pubertal timing since pubertal maturation differences in cognitive ability have shown varying results however, as stated above, sex differences are consistent after puberty. Some research has suggested that sex differences in cognitive ability emerge prepubertally although research is inconsistent (Maccoby & Jacklin,

1974; Newcombe, Bandura & Taylor, 1983; Linn & Peterson, 1985; Voyer, Voyer, & Bryden, 1995; Moore & Johnson, 2008; Quinn & Liben, 2008). At puberty however sex differences in verbal tasks become more prominent (Maccoby & Jacklin, 1974). In addition, at every age after puberty males consistently show higher spatial ability (Kerns & Berenbaum, 1991). Mental Rotations, which is a specific spatial test, consistently shows moderate to large (Cohen, 1992) effect sizes of sex differences favoring males (Vandenberg & Kuse, 1978; Linn & Petersen, 1985; Voyer, Voyer, & Bryden, 1995; Halpern, 2000; Resnick, 1993). Studying timing of puberty retrospectively in relation to cognitive ability will indirectly test the model of declining sensitivity in humans and may provide insight into the mechanism by which pubertal sex steroids influence behavior. This study will compare Waber's original hypothesis on cognition and pubertal timing with Sisk's more recent hypothesis on puberty as a declining period of sensitivity.

A comparison of Waber's and Sisk's past work is important in understanding the present study and hypotheses. Waber hypothesized and found that late maturing individuals performed better and early maturing individuals performed worse at mental rotations regardless of sex, indicating only pubertal timing affected cognitive ability (Waber, 1976). Sisk however, found that in hamsters, testosterone affected sex-typed behavior in hamsters that experienced early and on time puberty, indicating that behavior is influenced by pubertal timing and may differ depending on sex (Schulz, Molenda-Figueira & Sisk, 2009). According to Sisk, early maturing males would perform the best at spatial abilities when compared with on time maturing males, late maturing males, and all females. This may be due to the organizational effects of testosterone secretion during the onset of early puberty while the brain is most sensitive. Estrogen would not show organization effects because testosterone is the thought the leading

cause of sex differences in the Mental Rotations Test (Davison & Susman, 2001). There would be no difference in cognitive ability due to maturation rate among females since testosterone, driving the behavioral differences, is not the dominant hormone for females during puberty (Schulz, Molenda-Figueira & Sisk, 2009).

Verbal fluency, another cognitive task, shows a small (Cohen, 1992) female advantage over males (Maccoby & Jacklin, 1974; Hyde, 1981; Hampson & Kimura, 1988) and can be important in examining the influences of estrogen during puberty. Waber hypothesized and found that early maturing individuals performed the best at verbal tasks while late maturing individuals performed the worst regardless of sex (Waber 1976). Verbal fluency ability may be influenced by estrogen as a result of the female advantage, which would affect females more than males. Studies involving menstrual phases show how differing levels of estrogen can influence cognitive ability. In one study, women in the midluteal phase, categorized by increased estrogen, performed better at verbal fluency tasks than women in the menstrual phase, categorized by lower estrogen (Hampson, 1990). In addition, girls with Turners syndrome were found to have lower verbal fluency than control girls (Money & Alexander, 1965; Waber, 1979) Correlations between estrogen levels and verbal ability performance support the hypothesis that verbal fluency ability is estrogen-driven. According to Sisk, there should be no difference in verbal ability in males with differing maturation rates because estrogen is not the dominant hormone for males during puberty. Early maturing females however should perform the best at verbal fluency when compared to on time maturing females, late maturing females, and all males. These effects would support the hypothesis that early exposure to estrogen, due to early pubertal timing in girls during a peak sensitive period, will have greater organizational effects on the brain as seen by sex-typical behavior (Schulz, Molenda-Figueira & Sisk, 2009).

Method

Participants

Participants were recruited through the Undergraduate Psychology Subject Pool at The Pennsylvania State University. Participants were given a screening survey of pubertal maturation, in which the results were used to categorize them as early, on time, or late maturation. Participants were then contacted again and asked to take part a study of cognitive ability. The age range was 18-23 years with a mean (SD) of 19 .69 (1.01) years. There were a total of 139 participants: 64 females (early maturing females N=34, on-time maturing females N= 45, & late maturing females N=30) and 14 males (early maturing males N=6, on-time maturing females N=16, & late maturing males N=8).

Procedure

Pubertal Timing Categorization. Pubertal timing categorizes were determined by a two-part questionnaire with sex-appropriate questions. The first part included questions that asked individuals to recall the timing of their first menstrual bleeding for girls and wet dream for boys. The second part asked individual to recall certain events during puberty in relation to their peers. Participants had to record these events as much earlier, somewhat earlier, the same, somewhat later, or much later than their friends or peers (Appendix 1 & 2).

Online Survey. Participants completed an online survey through Surveymonkey.com, which was conducted in the lab under the supervision of a lab research assistant. The survey took about an hour to complete, and one credit for introductory psychology was given to

participants who successfully completed the survey. If only half of the survey was completed, half credit was given. Participants had the right the refuse to take part in the survey or withdrawal from the survey without penalty. The study was approved by the Institutional Review Board, IRB # 35739. Completion and submission of the survey meant the participant read and agreed to the online informed consent form.

Cognitive Measures. Cognitive measures were chosen because they show moderate-to-large (specific to sample) sex differences. The magnitude of the sex difference was reported in standard deviation units, represented by *d, d*= mean difference/average standard deviation. The Mental Rotation Test consisted of 20 items and required participants to mentally manipulate 3D depictions of block images. Participants were asked to choose the two of the four stimulus images that could be rotated in 3D space to produce same image as the target item. Participants were given ten minutes. Two points were possible per item so the maximum possible score was 40 (Vandenberg & Kuse, 1978).

A two part verbal fluency cognitive test was also used: In the first part, participants were given one minute to type words that described BLUE objects; in the second part, participants were given one minute to type words that have the same meaning as HAPPY (Peters, Reimers & Manning, 2006).

The Advanced Vocabulary test (Ekstrom et al., 1976) and was administered to asses general intellectual ability (Gouchie & Kimura, 1991). Subjects were shown a target word and asked to select one of five words under it with the same or nearly the same meaning. There were two parts of this test, with 18 questions each, so the maximum score was 36. Each part was allotted four minutes (Ekstrom et al., 1976).

Design

Pubertal Timing Categorization. Initial mass screening data was used to determine early, on time, and late pubertal maturation. Part II of the questionnaire was used as the primary bases for determination since Part I questions, which includes date of first menstrual cycle or first ejaculation, do not always correspond to onset of puberty (Hirsch et al., 1985). In addition, people are better at reporting retrospective data in regards to relative timing rather than specific dates (Kaiser & Gruzelier,1999; Koo & Rohan, 1997; Sanders & Soares, 1986). At first, pubertal groupings were based on the total mean score for all participants on the pubertal scale. The mean score represented the on-time maturing group while the mean score minus a standard deviation represented the early pubertal timing group and the mean score plus a standard deviation represented the late pubertal timing group. Individual mean scores from each participant were then calculated and an individual was grouped based on which category (early, on-time, or late maturing) their individual mean score fit with. This however resulted in a very small sample size for early and late maturing males (early maturing males N=2). To increase statistical power, the parameters which constituted early and late pubertal timing were expanded. Pubertal grouping was then based on the 5-point likert scale from the retrospective measure of puberty (1 indicating earlier pubertal timing and 5 indicating later pubertal timing) rather than using standard deviation units. A mean score of 1-2.4 was considered early, a mean score of 2.5-3.4 was considered on time, and a score of 3.5-5 was considered late. Individual mean score were then calculated and participants were grouped based on the parameters above.

Sex differences in cognition. Main effects of sex were analyzed from a two-way analysis of variance with factors of sex (male, female) and pubertal timing (early maturing, late maturing). The magnitude of the sex difference was reported in a standard deviation unit, *d*.

Mental Rotations tests have consistently shown one of the largest sex difference favoring males (Linn &Petersen, 1985; Halpern, 2000). Studies have shown fairly consistent effect sizes of *d* =0.73(Linn & Petersen, 1985), 0.90 (Masters & Sanders, 1993), 0.56 (Voyer, Voyer, Bryden 1995), and a range of 0.74-0.8 (reviewed in Resnick, 1993). In addition, studies have shown that around 20% of females score above average male scores in Mental Rotation tests (Bouchard & McGee, 1977; Harris 1978). It was therefore hypothesized that males will outperform females on Mental Rotations.

Verbal Fluency is a cognitive task where females outperform males (Hampson & Kimura, 1988). Effect sizes are small to moderate (Cohen, 1992) consisting of averages near *d*= 0.25 (Maccoby & Jacklin, 1974) and 0.24 (Hyde, 1981). Females were predicted to outperform males on Verbal Fluency.

A sex difference on the Advanced Vocabulary measure was tested by an independent t-test using participants of all pubertal groups. No sex differences were hypothesized for the Advanced Vocabulary measure which would be consistent with previous reports (Galea & Kimura, 1992; Kimura & Hampson, 1994).

Interactions between sex and maturation rate for cognition. Cognitive ability in relation to timing of puberty as well as sex was analyzed using a 2 (male and female) x 2 (early maturing, late maturing) analysis of variance (ANOVA) in order to specifically test the main hypotheses.

Research to date shows varying results of cognitive ability based on timing of puberty. If Waber's hypothesis is supported, it is expected that late maturers will perform the best in spatial ability(Figure 1), while early maturers will perform the best at verbal ability(Figure 2), regardless of sex (Waber, 1976). If Sisk's hypothesis is supported, it is expected that early maturing males will perform the best at spatial ability and there will be no difference in females (Figure 1). It would also be expected, according to Sisk, that early maturing females will perform the best at verbal ability, while there will be no difference in males (Figure 2) (Schulz, Molenda-Figueira & Sisk, 2009). Given the variability in the research that replicated Waber's work, results are expected to correspond with Sisk's hypothesis.

Results

Cognitive ability was examined with respect to pubertal timing and sex in order to compare two hypotheses about the effects of pubertal timing on brain sensitivity. Pubertal timing groups were determined by categorizing the participant's mean score from the four items on the retrospective measure of puberty. Each item was on a five-point scale. An individual mean score lower than 2.5 was considered early maturation while a mean score above 3.5 was considered late maturation. An analysis of variance was conducted to test main effects on each cognitive task of sex and pubertal timing and the interaction between sex and pubertal timing. Group differences are described by Cohen's *d, and by* 95% confidence intervals. Levene's Test for Equality of Variances was not significant for any analysis, $p>.05$, which indicates variances between groups in this study were not significantly different.

Sex Differences in Cognitive Ability

There was a significant sex differences in spatial ability, measured by Mental Rotations Test, were evident. Males performed significantly and substantially better than females, $F(1, 74) =14.535$, $p<.001$, as seen in Table 1. There was not a significant sex difference in verbal fluency ability, $F(1, 74) = .850$, $p>.05$ as shown in Table 1. There was not a significant sex difference on the Advanced Vocabulary measure, $F(1,74)=.734$,$p>.05$.

Pubertal Timing differences in Cognitive Ability

Results indicate no significant pubertal timing differences for either cognitive task: Mental Rotations: $F(1,74)=.322$, $p>.05$; Verbal Fluency: $F(1,74)=.896$, $p>.05$.

Sex and Pubertal Timing Interactions

A significant interaction was found between sex and pubertal timing on Verbal Fluency, $F(1,74)= 5.582$, $p<.05$. As shown in Table 1, late maturing males had the best verbal fluency score and early maturing males had the worst. Early maturing females performed better than late maturing females but worse than late maturing males on the Verbal Fluency task. No significant interaction between sex and pubertal timing was detected for Mental Rotations, $F(1,74)= 1.538$, $p>.05$.

Discussion

The purpose of this study was to examine the effects of pubertal timing on cognitive ability. Cognitive data from young adults were used to test two different hypotheses about pubertal timing effects on cognition. If Waber's hypothesis was supported, there should be an effect of pubertal timing on cognitive ability that would be the same in both sexes, with early maturers showing better verbal ability and late maturers showing better spatial ability. If Sisk's hypothesis was supported, there should be an interaction between sex and pubertal timing on cognitive ability with early pubertal maturers acting more sex-typical on specific cognitive tasks. According to Sisk's hypothesis, early maturing females should have the best verbal ability while early maturing males should have the best spatial ability.

Results indicated a main effect of sex for Mental Rotations which was predicted and consistent with previous findings (Linn &Petersen, 1985; Halpern, 2000; Voyer, Voyer, Bryden 1995; Masters & Sanders, 1993). Unlike previous studies, there was no significant sex effect for verbal fluency (Maccoby & Jacklin, 1974; Hyde, 1981; Hampson & Kimura, 1988) indicating a possible limitation of the measure or statistical power, as discussed below. As predicted, and consistent with previous results (Galea & Kimura, 1992; Kimura & Hampson, 1994), there was no sex effect for Advanced Vocabulary.

Neither Waber's nor Sisk's hypothesis was fully supported. With regard to Waber's hypothesis, there was no significant main effect of pubertal timing on any cognitive measure. Results indicate that the two pubertal groups, early maturers and late maturers did not significantly differ and results differed within each sex. Follow up studies of Waber's original work have varying results, with some studies reporting no significant links between cognitive

ability and maturation rate (Strauss & Kinsbourne, 1981; Waber et al., 1985; Newcombe, Dubas & Baenninger, 1989), similar to the results of this study. Other studies, however, did successfully replicate Waber's original findings(Sanders & Soar, 1986).

With regard to Sisk's hypothesis, no interaction of sex and pubertal timing was found for Mental Rotations. Results on verbal ability partially support Sisk's hypothesis because early maturing females performed better than late maturing females on verbal fluency however, an unexpected significant interaction was found. Late maturing males performed better at verbal fluency than early maturing males, which is inconsistent with Sisk's hypothesis.

There are some possible explanations for the failure to find predicted effects on Mental Rotations and Verbal Fluency. With regard to Mental Rotations, there may not be a window of declining brain sensitivity to testosterone and instead, sensitivity is constant across puberty. This would therefore mean that everyone, regardless of pubertal timing, is influenced by increasing testosterone at puberty, with males showing higher levels of testosterone and females showing lower levels of testosterone. This is not likely a mechanism however since a sex difference in Mental Rotations Test appears in pre-pubertal children, indicating pubertal hormones are not the only factor that gives rise to sex differences in spatial ability (Vederhus & Krekling, 1996).

It may be that verbal ability, which is thought to reflect the organizational effects in the brain due to estrogen (Sherwin, 2002), reflects activational effects of the brain. Previous studies involving menstrual cycles show that verbal fluency ability was found to be positively correlated with estrogen levels during the menstrual cycle (Hampson, 1990). Studies of post-menopausal women also show better verbal fluency in females with estrogen treatment compared to women

not on estrogen treatment (Sherwin, 2002). These results indicate that verbal fluency is likely estrogen driven but reflect activational effects on the brain.

It is also possible that Sisk's theory, first developed in rodent models, is not applicable to human beings, which therefore may explain non-significant effects and unexpected interactions between sex and pubertal timing for cognitive ability. Sanders and Soares, who studied sexual maturation in regards to spatial ability, suggested that pubertal timing and sex may independently contribute to spatial ability, which may explain the lack of interactions in the present study (Sanders & Soares, 1986).

There are several limitations of the study that must be taken into consideration when interpreting the results. The first is the measure of pubertal timing. Pubertal data were retrospectively self-reported and the measure asked participants to indicate timing of certain pubertal events in comparison to others on certain events. Retrospective reports of perceptions of pubertal timing have previously been reported to be valid by comparing them to explicit dates when pubertal markers occurred (such as date of first menstruation. It is therefore unlikely that this measure was not sensitive enough (Dubas, Graber, & Peterson, 1991). It is, however, possible that the grouping of pubertal timing affected results. Grouping of pubertal timing for early and late puberty was completed by grouping individual mean scores from the pubertal measure for each participant into early and late parameters based on a 5- point puberty scale, rather than using standard deviations. This expanded the sample size for early and on time individuals but may have resulting in improper categorization, which then may have made it difficult to see expected effects. For example, Diamond, Carey, and Back (1983) found that early maturers showed higher spatial ability than late maturers. Findings were attributed to extreme

groupings (Diamond, Carey, & Back, 1983). In this study, the lack of extreme groupings due to widened parameters may have caused too much within-group variability. Alternatives to increase between group variability resulted in insufficient sample sizes and were therefore not used.

The lack of expected results may also be attributed to a relatively small sample size which resulted in low statistical power. It is interesting that the means for the Mental Rotation Test are in the expected direction of Sisk's predicted hypothesis. Large confidence intervals of mean differences suggest that with a larger sample size and greater statistical power, significant results may be evident.

In addition, the specific fluency tasks may not have been sensitive enough to detect a sex effect. Although most research report observed sex differences, some studies have not been able to find significant sex differences in verbal fluency ability (Maccoby &Jacklin, 1974). Most studies report a small effect size with a median report of .24 (Hyde, 1981). Furthermore, if in fact the difference in population means between males and females is in fact small, then some repeated studies may not find significant differences (Hyde, 1981). The absence of significant sex effects in this study therefore is most likely is a result of low statistical power.

Further research is needed to examine effects of sex hormones on the brain during puberty. Follow up research should replicate this study but increase the sample size and thus increase the statistical power. In order to test whether sex hormones released during puberty have organizational effects, a longitudinal study should be conducted which assesses cognitive ability during puberty and again in adulthood to determine if differences are lasting. In addition, to test whether there is a period of declining brain sensitivity to sex hormones during puberty, studies should be conducted on individuals with clinically atypical pubertal timing, rather than normal

variations in pubertal timing. Specifically, girls with precocious puberty, where the release of estrogen, and therefore the onset of puberty occurs extremely early (Ehrhardt, & Meyer-Bahlburg, 1986), would be a good sample to examine the effects of on cognition. There is little research on cognitive ability in girls with precious puberty. Most of the research on girls with precious puberty has not been able to detect an increase in verbal ability but has been able to find a decrease in spatial ability (Ehrhardt, & Meyer-Bahlburg, 1986; Meyer-Bahlburg et al., 1985; Rovet, 1983).

In conclusion, the findings from this study do not fully support either Waber's hypothesis (only a pubertal timing effect on cognitive ability) or Sisk's hypothesis (pubertal timing and sex interaction on cognitive ability) regarding the influence of sex hormones and pubertal timing on cognitive ability. In this study, maturation rate was not seen to have an effect on cognitive ability. Future work should be conducted on the theory of declining brain sensitivity to sex hormones to examine whether certain trends seen in this study, specifically the interactions of sex and pubertal maturation on spatial ability, truly reflect differences in the population.

Figure 1

A comparison of Waber and Sisk's hypotheses regarding pubertal timing influences on spatial ability.

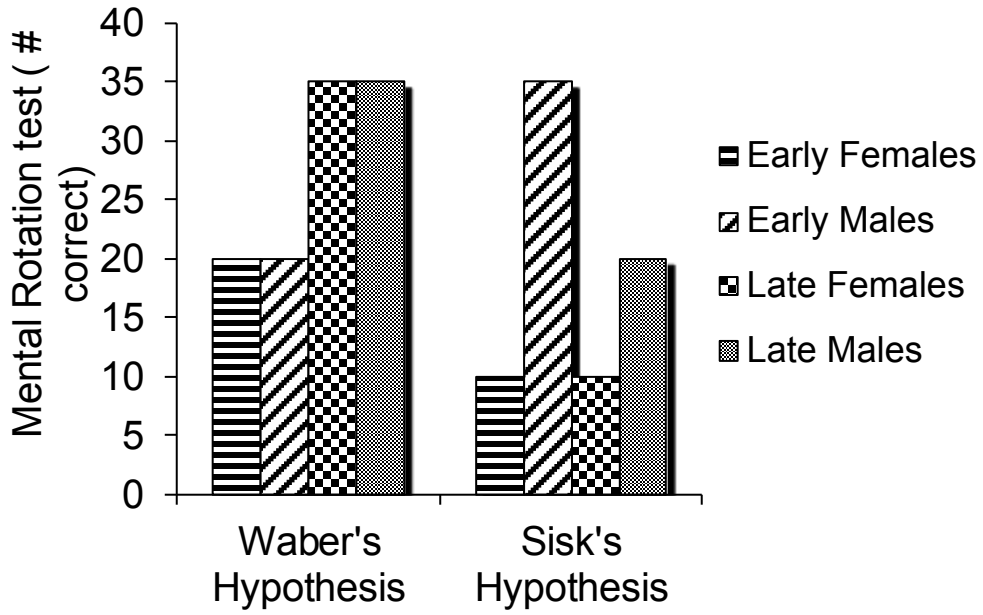

Figure 2

A comparison of Waber and Sisk's hypotheses regarding pubertal timing influences on verbal ability.

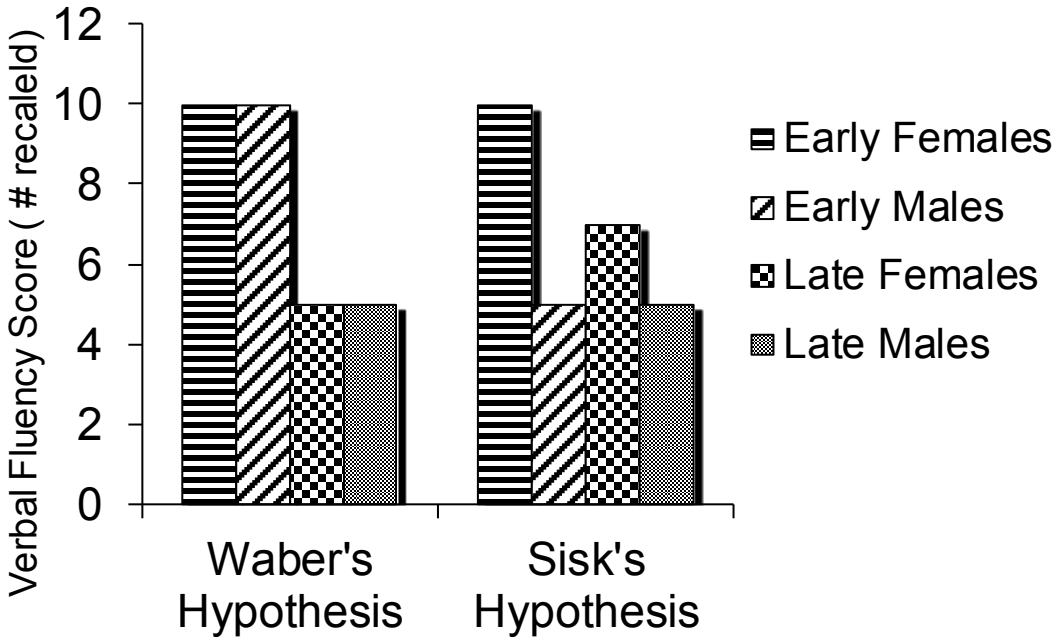

Table 1

Mental Rotation and Verbal Fluency ability in males and females of different pubertal timing

	Females				Males					
	Early N=34	Late N=30	*d*	Total N=64	Early N=6	Late N=8	*d*	Total N= 14	*Sex Difference d*	95% CI of Mean Sex Difference
Mental Rotations Test	28.41 (5.15)	29.47 (5.51)	0.20	28.91 (5.30)	36.33 (2.94)	33.5 (6.05)	0.60	34.71 (5.01)	1.13*	2.72, 8.89
Verbal Fluency	7.29 (2.97)	6.23 (1.92)	0.43	6.80 (2.57)	4.83 (2.38)	7.31 (2.43)	0.20	6.25 (2.64)	0.13	-2.07, .972

*Sex differences tested by a two way ANOVA were reported as significant, p<.001

Appendix A

Mass Screening Questionnaire for Females.

Retrospective Report on Puberty (Female)

These questions are about your physical development during your early adolescence, particularly changes at puberty. For some questions, we ask about the specific age at which an event happened, whereas for other questions, we ask about how your development compared to that of your friends. For all of the questions, it may help you to form a general image of yourself during your early teenage years. Think about the house you lived in, the school you attended, things you generally did during the day, and your favorite friends. It may also help you to think about the kinds of things that were happening at the time, such as what season it was (for example, what was the weather like? were you in school?) or holidays that might have occurred nearby (for example, was it close to your birthday? Was it close to Thanksgiving?) You do not have to report any of these things about yourself, just keep the images in mind as you answer the following questions.

Part I

A. As best you can remember, how old were you when you had your first period (that is, your first menstrual bleeding)? _____ years _____ months

Part II

Sometimes it is difficult to remember exact dates. The following questions ask about timing relative to those around you rather than asking about a specific date. For each item, consider how the timing of your experience compared to that of your friends. Please rate your response according to the five-point

		Much Earlier	Somewhat Earlier	The Same	Somewhat Later	Much Later
A.	Having your first period	1	2	3	4	5
B.	Having noticeable breast development/growth	1	2	3	4	5
C.	Experiencing your teenage growth spurt	1	2	3	4	5
D.	Buying your first bra	1	2	3	4	5

Appendix B

Mass Screening Questionnaire for Males.

Retrospective Report on Puberty (Male)

These questions are about your physical development during your early adolescence, particularly changes at puberty. For some questions, we ask about the specific age at which an event happened, whereas for other questions, we ask about how your development compared to that of your friends. For all of the questions, it may help you to form a general image of yourself during your early teenage years. Think about the house you lived in, the school you went to, things you usually did during the day, and your favorite friends. It may also help you to think about the kinds of things that were happening at the time, such as what season it was (for example, what was the weather like? were you in school?) or holidays that might have occurred nearby (for example, was it close to your birthday? Was it close to Thanksgiving?) You do not have to report any of these things about yourself, just keep the images in mind as you answer the following questions.

Part I

A. As best you can remember, how old were you when you had your first "wet dream" (that is, your first ejaculation during sleep)? _____ years _____ months

Part II

Sometimes it is difficult to remember exact dates. The following questions ask about timing relative to those around you rather than asking about a specific date. For each item, consider how the timing of your experience compared to that of your friends. Please rate your response according to the five-point

		Much Earlier	Somewhat Earlier	The Same	Somewhat Later	Much Later
A.	Having your first wet dream	1	2	3	4	5
B.	Growing a beard/Needing to begin shaving	1	2	3	4	5
C.	Experiencing your teenage growth spurt	1	2	3	4	5
D.	Changing/Cracking of your voice	1	2	3	4	5

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Academic Vita

AMY DANIELLE KAPLAN

EDUCATION

The Pennsylvania State University, Schreyer Honors College — **University Park, PA**
Candidate for Bachelors of Science in Psychology: Neuroscience — *Class of May 2012*

Eastern Regional High School — **Voorhees, NJ**
High School Diploma — *Class of 2008*

RESEARCH

Dr. Sheri Berenbaum's Lab — **University Park, PA**
Research Assistant — *June '10-Present*

- Analyze data on individuals with Congenital Adrenal Hyperplasia and Androgen Insensitivity Syndrome
- Conduct special projects on occupational interest and cognitive and spatial ability in adolescents
- Evaluate and input data in SPSS Data Entry

Honors Thesis Research — *June '10-Present*

- *Cognitive ability in Young Adults: Testing Hypotheses on Declining Brain Sensitivity to Pubertal Hormones*
- Evaluated cognitive ability with regard to timing of pubertal maturation and sex

Pennsylvania State University Undergraduate Research Exhibition — **University Park, PA**

- Selected to present research posters on: *April 2011*
  - Attitudes Towards Adult Entertainment

Psi Chi Research Conference — **University Park, PA**

- Selected to present research posters on: *April 2012*
  - *Cognitive ability in Young Adults: Testing Hypotheses on Declining Brain Sensitivity to Pubertal Hormones*

TEACHING EXPERIENCE

Introduction to Biology — **University Park, PA**
Teaching Assistant — *Aug '10 – Present*

- Conduct a lab for 25 students once weekly, administer weekly quizzes, and facilitate lab procedures
- Maintain quality control of the course for future semesters

Peer Tutor — *June '10 – Present*

- Assisted students in a weekly tutor session

SERVICE

Global Medical Brigades — **University Park, PA**
Fundraising Chair — *Aug '08 – Present*

- Organize fundraising efforts on campus to raise over $10,000 in the Fall '10 semester

Honduras Brigade — *March '10*

- Provided treatment for over 1,800 patients over a spring break volunteer trip to Honduras
- Shadowed doctors, worked in triage, and assisted with pharmaceutical distribution

Ghana Brigade — **December 2011*

Trilogy, founding member — **University Park, PA**
Fundraising Trip Coordinator — *Aug '10 – Present*

- Organize fundraising trips for 160 people to raise money for pediatric cancer through the Penn State Dance MaraTHON

Fresh Start Fall 2008 — **University Park, PA**

- Campus wide community service effort

Urban Service Experience **University Park, PA**

- Volunteered at a soup kitchen and planted a garden in Philadelphia, PA *Summer '08*

WORK EXPERIENCE

Virtua Health Hospital **University Park, PA**

Summer Intern *Summer 2009*

- Shadowed a general surgeon, osteopathic surgeon, and the Director of the Intensive Care Unit
- Analyzed data from the quality and risk management department
- Worked with the Patient Representative to assist with patient overflow during flu epidemic

Margate Dairy Bar **Margate, NJ**

Summer2007-2009

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References available upon request