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Pubertal Testosterone Predicts Mental Rotation Performance of Young Adult Males

Eero Vuoksima^{1,2,*}, Jaakko Kaprio^{1,3,4}, C. J. Peter Eriksson¹, and Richard J. Rose^{1,5}

¹Department of Public Health, University of Helsinki, Helsinki, Finland ²Department of Psychiatry, University of California, San Diego, La Jolla ³Finnish Institute for Molecular Medicine, University of Helsinki, Helsinki, Finland ⁴Department of Mental Health and Substance Abuse Services, National Institute for Health and Welfare, Helsinki, Finland ⁵Department of Psychological and Brain Sciences, Indiana University, Bloomington, USA

Summary

Robust sex differences in some spatial abilities that favor males have raised the question of whether testosterone contributes to those differences. There is some evidence for prenatal organizational effects of testosterone on male-favoring spatial abilities, but not much is known about the role of pubertal testosterone levels on adult cognitive abilities. We studied the association between pubertal testosterone (at age 14) and cognitive performance in young adulthood (at age 21–23), assessing male-favoring, female-favoring, and sex-neutral cognitive domains in a population-based sample of 130 male and 178 female twins. Pubertal testosterone was negatively associated with performance in the Mental Rotation Test in young adult men ($r = -.27$), while among women no significant associations between testosterone and cognitive measures were detected. The significant association among men remained after controlling for pubertal development. Confirmatory within-family comparisons with one-sided significance testing yielded a negative correlation between twin pair differences in testosterone levels and Mental Rotation Test performances in 35 male twin pairs ($r = -.32$): the twin brother with higher testosterone performed less well on the Mental Rotation Test. That association was evident in 18 pairs of dizygotic male twin pairs ($r = -.42$; analysis controlling for shared environmental effects). In contrast, the association of differences was not evident among 17 monozygotic male twin pairs ($r = -.07$; analysis controlling for shared genetic influences). Results suggest that pubertal testosterone levels are related specifically to male-favoring spatial ability and only among men. Within-family analyses implicated possible shared genetic effects between pubertal testosterone and mental rotation ability.

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*Corresponding author: Eero Vuoksima, Department of Psychiatry, University of California, San Diego, 9500 Gilman Drive (MC 0738), La Jolla, CA 92093-0738, USA. tel. + 1 858 534 8269, fax: + 1 858 822 5856, eero.vuoksima@helsinki.fi.

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Contributors

EV created the study hypothesis, managed the literature searches, undertook statistical analyses, wrote the first draft of the manuscript, and supervised and participated in the collection of neuropsychological test data. CJPE determined hormonal measures. JK and RJR designed the Finn Twin 12 study protocol and were responsible for data collection. All authors contributed to and have approved the final manuscript.

Keywords

Mental Rotation Test; Pubertal development; Sex difference; Spatial ability; Testosterone

Introduction

On average, males outperform females in some domains of spatial abilities. One such domain is mental rotation ability; the widely used Vandenberg and Kuse Mental Rotation Test (Vandenberg and Kuse, 1978) yields a large and robust sex difference with male mean scores about one full standard deviation higher than those of females (Voyer et al., 1995). Studies have investigated the hormonal basis in male favoring spatial abilities and have suggested a role for testosterone in creating these sex differences. The actions of testosterone on brain are usually considered to be either organizational or activational in nature (see e.g. McCarthy & Arnold, 2011). Organizational effects refer to permanent brain programming effects of testosterone during developmental stages of brain sexual differentiation, usually occurring during perinatal development, while reversible activational effects refer to temporary changes in brain and behavior activated by circulating testosterone levels at any time of life.

There has been a long-standing question of whether testosterone has sex specific effects on male favoring spatial abilities. Some studies have indicated a positive correlation between testosterone and male favoring spatial abilities in females, but a negative correlation among males, which raise the question of whether optimal levels of testosterone are requisite for good spatial abilities (Shute et al., 1983; Gouchie and Kimura, 1991; Moffat and Hampson, 1996). Nevertheless, other studies have reported a positive correlation between male favoring mental rotation ability and testosterone levels among males, as well (e.g. Silverman et al., 1999; Hooven et al., 2004;). Recently, Puts et al. (2010) reviewed most, but not all studies of testosterone - spatial ability relationships, including mostly studies on young adults. They evaluated the relationship between male favoring spatial abilities and testosterone levels measured at the time of administration of spatial tests and concluded that there is no consistent evidence that testosterone has activational effects on spatial abilities (Puts et al., 2010). Further support for this conclusion was also reported in the same study by Puts et al. (2010), in which no significant association between activational testosterone levels and mental rotation performance was observed in a large new sample of young adults comprised of 160 females and 177 males.

Among elderly, testosterone substitution can enhance the performance in the domain of spatial abilities (see e.g. Beauchet, 2006). For example, improving effects of testosterone supplementation on spatial memory have been reported both among healthy men (Cherrier et al. 2001) and men with mild cognitive impairment and Alzheimer's disease (Cherrier et al., 2005). However, contradictory results have emerged among non-clinical samples of adult men over 35 years of age: two large-scale studies have indicated both negative (Yonker et al., 2006; n = 450) and positive (Thilers et al., 2006; n = 1107) associations between testosterone and visuo-spatial abilities.

As testosterone levels are highly heritable throughout life (Eriksson et al., 2005; Ring et al., 2005; Hoekstra et al., 2006; Kuijper et al., 2007), it is difficult to discriminate whether the testosterone levels measured in one time point reflect temporary activational or permanent organizational effects of testosterone. Different measures of spatial abilities and different types of hormone samples (saliva vs. blood) may also explain inconsistent results across studies.

Puts et al. (2010) have suggested that if there are effects of testosterone on spatial abilities, the timing of these effects must stem from the organizational effects that occur during perinatal or pubertal development when testosterone levels are highest before adulthood. Concerning organizational effects of testosterone, there is some evidence that the prenatal levels of testosterone are related to spatial abilities. Meta-analysis of individuals with congenital adrenal hyperplasia (CAH), a clinical condition exposing the fetus to excessive amounts of testosterone, offer support for sex-specific effects of testosterone on spatial abilities (Puts et al., 2008). Females with CAH had better spatial abilities than non-CAH individuals, and males with CAH were inferior to non-CAH individuals in their spatial abilities. Also, a recent twin study reported significantly better mental rotation test performance in females with a male co-twin compared to females with a female co-twin (Vuoksimaa et al., 2010). Having a male co-twin may be a possible source of prenatal exposure to higher levels of testosterone in females, although this result might also reflect postnatal socialization effects. Another twin study replicated this masculinization of mental rotation ability among females with a male co-twin, but found no masculinization among non-twin females with a slightly older brother compared to non-twin females with slightly older sister (Heil et al., 2011). These results were interpreted as support for the prenatal effects of testosterone rather than postnatal socialization effects. Finally, the relationship between the ratio of second and fourth digits (2d:4d, a putative marker of prenatal testosterone levels) and spatial abilities have been examined, but no consistent association has emerged neither in females or males (see Puts et al., 2008 for a meta-analysis). However, a large scale internet study of > 200,000 participants (also included in the meta-analysis of Puts et al. 2008) found that low 2d:4d ratio (as a proxy for higher prenatal testosterone levels) was associated with better mental rotation performance both in females and males (Peters et al., 2007). Studies on transsexuals have yielded support to both organizational (van Goozen et al., 2002) and activational (Slabbekoorn et al., 1999) effects of testosterone on spatial abilities.

Beyond prenatal developmental effects, testosterone may have long lasting effects on cognitive functioning during pubertal development, a time when there is a substantial peak in testosterone production, especially among boys (Hiort, 2002). Sex hormones may have organizational effects on the developing human brain during pubertal development (for reviews, see Sisk and Zehr, 2005; Hines, 2011; Peper et al., 2011), and animal studies have suggested that it might be the last developmental stage in which brain organization is sensitive to sex hormones (Schulz et al., 2009).

To our knowledge, no large population-based studies have investigated the effects of pubertal testosterone levels on cognitive abilities in young adulthood. However, some research on the effects of pubertal development on cognitive abilities in adolescence does exist. Some 30 years ago, a group of studies investigated whether the rate of maturation during puberty has effects on cognition. A rationale for these studies stemmed from the observed cognitive sex differences during and after puberty and from the fact that pubertal maturation occurs ~1.5 – 2 years earlier in girls than in boys. Rate of physical maturation during puberty has been related especially to spatial abilities. Late maturers have been reported to have better spatial skills compared to early maturers among both adolescent boys and girls (Waber, 1976; Waber, 1977; Newcombe & Bandura, 1983). In contrast, several studies have reported no relationship between the stage of pubertal development and spatial abilities (e.g. Newcombe et al., 1989). While the rate of maturation during puberty may affect the spatial abilities, the underlying mechanism is unclear. Cerebral organization of the brain (Waber, 1976) and psychosocial factors, including participation in spatial activities (Newcombe & Bandura, 1983), and socioeconomic background (Waber et al., 1981) have been suggested to explain the association between rate of maturation and spatial abilities

during puberty. But could the association be explained by pubertal testosterone levels? Unfortunately, not many studies have tested this.

Based on experimental study of sex steroids in adolescents who were treated for delayed puberty, Liben et al. (2002) concluded that spatial abilities are not greatly affected by sex hormones during puberty, although this could not be generalized to normal developing adolescents. One study of 9 – 14 year boys and girls, representing individuals from all pubertal development stages, measured testosterone levels from blood samples taken in three time points with six month intervals (Davison & Susman, 2001). This study showed that among boys, testosterone had a positive association with spatial abilities (mental rotation and block design performance) but not with verbal ability at each time. Among girls, mental rotation performance was positively associated with testosterone levels only at the last assessment. The stage of pubertal maturation did not have any relationship with spatial or verbal abilities neither in boys or girls (Davison & Susman, 2001). Another longitudinal study of adolescents tested the association between testosterone levels and spatial abilities, but found no consistent evidence for any pattern of relationship (Hassler, 1991; Hassler et al., 1992). To date, no studies have tested the association between pubertal testosterone levels and cognitive abilities in adulthood. One study of females tested whether the effect of late maturation on cognition persist into adulthood: the effect of maturation rate was not evident any more on adult spatial abilities (Strauss & Kinsbourne, 1981).

The aim of our study was to test the association between pubertal levels of salivary testosterone and cognitive abilities in young adulthood. We tested whether the effect of testosterone was specific to male-favoring cognitive ability. Also the direction, as well as possible sex-specificity, of the association were questions of interest.

Methods

Participants

We studied the association between pubertal testosterone levels and cognitive abilities in young adulthood among twins from the longitudinal population-based FinnTwin12 study comprising Finnish twins born in 1983 – 1987 (Kaprio et al., 2002). The present analysis included all twins from the 1986 and 1987 birth cohorts who had both testosterone data at 14 years and cognitive data at 21 – 23 years (testosterone assays were available only for twins born in 1986 and 1987; no other selection criterion than cohort year was used for collecting the saliva sample). The sample of 308 eligible participants included 130 men and 178 women. There were 134 twin pairs and 40 individual twins without their co-twin. The full pairs included 29 monozygotic (MZ) female pairs, 17 MZ male pairs, 30 dizygotic (DZ) female pairs, 18 DZ male pairs, and 40 DZ opposite-sex pairs. Zygosity was determined from DNA for all 94 same-sex pairs.

All participants gave written informed consent before participating in neuropsychological testing at ages 21 – 23. Both twins and their parents gave consent for the twins' participation at age 14. The study protocol for FinnTwin12 was approved by the ethical committee of Helsinki and Uusimaa hospital district, Finland and the Institutional Review Board of Indiana University, Bloomington, USA. Twins received a gift certificate worth 40 euros as a compensation for their participation in the one-day laboratory testing protocol at ages 21 – 23, and a gift certificate worth 5 euros was given at age 14.

Cognitive measures

Neuropsychological tests were administered as a part of one-day laboratory testing protocol in the Department of Public Health, University of Helsinki. A total of 812 twins completed a series of individual and group administered neuropsychological tests: for 308 of these twins

we also had testosterone measured at age 14, and they form the sample for our analysis. The Mental Rotation Test, Object Location Memory Test, and Digit Symbol were administered in a group test format for most of the subjects (testing sexes separately, there were no differences between participants who completed these tests as individually administered and participants who completed these tests as a group format, all p-values for differences between groups were larger than .40), while Digit Span was administered as an individual test for all participants. There were no differences in distributions on any of these cognitive measures between twins for whom we had testosterone measured ($n = 308$) and those for whom we did not ($n = 504$) (testing sexes separately, all p-values for differences between twins with testosterone data and twins without testosterone data were greater than .11, except a trend for better MRT performance among women who had testosterone data ($M = 9.87$, 95% confidence intervals, 9.14 – 10.59) compared to women who did not have testosterone data ($M = 9.00$, 95% confidence intervals 8.47 – 9.54, $p = .06$).

We used the redrawn version of Vandenberg and Kuse Mental Rotation Test (MRT) to assess a male-favoring spatial ability (Peters et al., 1995). The task required participants to mentally rotate two-dimensional drawings of three-dimensional objects through the vertical axis. Each trial contained one target figure and four figures that represented the target figure viewed from different angles. In each trial, two of the four figures could be mentally rotated to match the target figure. One point was given if the participant marked both correct figures, yielding a possible MRT score range from 0 to 24. The MRT was administered in two parts, each consisting of 12 trials. The time limit for each part was three minutes with a one-minute break between the two parts. A five-minute practice period including four practice trials was administered before the actual test.

Object Location Memory (OLM) Test (Silverman and Eals, 1992) was used to measure a female-favoring spatial ability. In this test, participants were shown drawings of 27 objects for a period of one minute with instruction to memorize all objects. After one minute, they were shown another set of drawings in which the same 27 objects appeared but in which 14 objects had changed their location. The subject's task was to identify which objects had changed their location and which had not. One point was given for correct answers and one point was deducted for incorrect answers. Participants had one minute to indicate whether the items had changed or retained their location. The possible total score for OLM ranged from - 27 to 27. We excluded one participant with a negative score (presumably due to misunderstanding of task instructions) from the analyses to yield a normal distribution of OLM scores ranging from 1 to 27 in our data (excluding participant with negative score had no effect on the correlations between OLM and other measures).

Processing speed was studied with the Digit Symbol subtest from the Wechsler Adult Intelligence Scale III (WAIS-III; Wechsler, 1997). Participants copied symbols that matched numbers from one to nine as fast as possible. There was a two-minute time limit and a brief practice with four items before the test. The number of correctly marked symbols was used as a measure of processing speed. Digit Symbol was considered as female favoring non-spatial cognitive measure: according to Finnish WAIS-III manual the effect of sex is 0.54 as indexed by Cohen's d (Wechsler, 2005).

Auditory working memory was studied with the Digit Span subtest from the Wechsler Memory Scale – Revised (WMS-R; Wechsler, 1987). In the forward condition of Digit Span, participants had to repeat the span of numbers in the same order as they were read aloud by the experimenter. In the backward condition, participants had to repeat the span of numbers in the reverse order, from the end to start. The length of a span increased by one number after two trials with the same span length. Forward condition started with a span consisting of three digits and backward condition started with a span consisting of two

digits. Participants received one point for each correctly completed span. The number of correctly completed spans was used as a measure of auditory working memory. Digit Span was considered as a sex-neutral cognitive measure (e.g. Latvala et al. 2009, Torniainen et al. 2011).

Testosterone measure

Pubertal testosterone at age 14 years was determined from two saliva samples. The saliva samples were collected in conjunction with an interview, the first sample before and the second sample afterwards. Testosterone concentration was determined from the saliva supernatants with ^{125}I radioimmunoassay kits from Orion Diagnostica, Espoo, Finland. The inter-assay variability was 9.7%, and intra-assay variability was 6.2%, at the level of 0.13 nmol/L ($n=25$). The testosterone measure used in all analyses was a log transformed mean of two samples adjusted for time of the day and seasonal variability. Log transformation was performed for reaching normal distribution. Both testosterone measures were available for 303 participants, but in five participants adjusted log transformed testosterone measure was based only on the first saliva sample due to missing data on the second sample. Testosterone assays were available for a total of 875 twins at age 14: for 308 of these twins we also had cognitive measures at ages 21–23. There were no significant differences in testosterone levels between twins who did and twins who did not have cognitive data in young adulthood (tested separately among males: $F(1, 295) = 0.02$, $p < .88$; and females: $F(1, 271) = 1.52$, $p < .22$). See Eriksson et al. (2005) for details in the saliva sample collection and determination of testosterone levels.

Pubertal development measure

We used the 5-item Pubertal Development Scale, PDS (Petersen et al., 1988) included as part of a longer questionnaire that twins filled out at age 14, to assess the stage of the pubertal development. The questions in PDS concern physical development with two sex-specific questions. Four-point scale was used to rate the developmental stage in each item (1 = no development, 2 = development has just begun, 3 = development is definitely underway, and 4 = development is complete). The PDS can be considered as reliable instrument (median alpha coefficient of .77 reported in Petersen et al., 1988) that correlates with physician rating (Brooks-Gunn et al., 1987). The means for all items of PDS in Finn Twin 12 have been reported to be slightly lower than in the American samples (Dick et al., 2001), but the reliability was adequate both for boys (alpha .67) and girls (alpha .56) as reported in Dick et al. Data on PDS were available for 124 men and for 165 women.

Statistical analyses

Our analytic strategy included both between-family (of all twin individuals) and within-family (testing within-pair differences of twin pairs) comparisons. In between-family analyses, we tested whether there were significant associations between pubertal testosterone and cognitive measures. All significant associations obtained in between-family analyses were further tested in within-family analyses. These analyses tested whether the within pair differences in testosterone levels are related to within pair differences in cognitive ability. Testing between-family associations in within-family comparisons is a powerful strategy to control for unmeasured between-family environmental confounds while controlling also for genetic variance, completely within MZ twin pairs and partly within DZ twin pairs (see Dick et al., 2000). In these analyses, one sided p-values were used, because we expected that the relationships of testosterone levels to cognitive measures would be in the same direction within twin pairs as observed in between-family analyses of twins as individuals; that is for example, if higher levels of testosterone were associated with better cognitive performance in twins as individuals (between-family comparison), the co-twin

with higher testosterone would be expected to be the co-twin with better cognitive performance (within-family comparison).

We also used regression analyses to study the effects of covariates in the associations between pubertal testosterone levels and cognitive performance. The non-independence of clustered data (twins nested in families) was taken into account: the family was included as a cluster variable in the regression analyses to obtain robust standard errors (Williams, 2000). In the first stage, twin data were analyzed at individual level (between family-analyses). We then tested whether the associations obtained from between family-analyses remained in within-family analyses. All analyses were carried out with Stata 11.0 statistical software (StataCorp, College Station, TX, USA).

Results

Expected sex differences in the mean levels of testosterone, pubertal development, and in cognitive measures were detected (Table 1). Based on Wald test, there was no significant difference ($F(1,173) = .06$, $p = .81$) in the age at neuropsychological testing between men ($M = 21.87$, $SD = .40$) and women ($M = 21.88$, $SD = .37$). As indexed by χ^2 -test, there were significantly ($F(1,175) = 4.25$, $p < .05$) more women (77%) with high school education compared to men (65%). We tested the effects of age at neuropsychological testing and education (having a high school diploma vs. no high school diploma) on cognitive measures in a series of regression analyses with sex as a covariate. Age had no significant effect on any of the cognitive measures (all p -values higher than .18). The level of education was a significant predictor of Digit Symbol ($F(2,173) = 13.93$, $p < .001$, partial $\eta^2 = .09$) and Digit Span performance ($F(2,173) = 9.59$, $p < .001$, partial $\eta^2 = .09$) indicating that participants with high school education performed better than less educated participants in these tests. Level of education did not have a significant effect on MRT ($p = .36$) and OLM ($p = .14$) performance, and similarly, education was not related to MRT and OLM when males and females were analyzed separately. The sex differences in Digit Symbol, MRT, and OLM performance remained in regression analyses with education as a covariate.

Linear regression analysis ($F(3, 166) = 66.45$, $p < .001$, $R^2 = .45$; Fig. 1) showed that sex ($p < .05$) was significant predictor of testosterone levels while pubertal development had a non-significant effect ($p = .32$) on testosterone levels. However, there was a significant interaction between PDS and sex ($p < .001$) indicating that the testosterone levels were associated with pubertal development only in males (Fig. 1). Testosterone levels were similar among males and females whose pubertal development had not begun, while the sex difference in testosterone levels increased as a function of advanced pubertal development.

Between-family analyses of the associations between pubertal testosterone and cognitive measures

There was a negative correlation between pubertal testosterone and MRT performance in adult men ($r = -.27$, $p < .01$, Fig. 2A), but not in women ($r = .02$, $p = .83$, Fig. 2B). No other cognitive measures correlated with pubertal testosterone in either men or women (Table 2). Linear regression analysis ($F(3,171) = 14.39$, $p < .001$, $R^2 = .14$) indicated that the sex-testosterone interaction term was a statistically significant predictor of MRT performance ($t = -2.26$, $p < .05$, partial $\eta^2 = .03$). Given the significant sex-testosterone interaction and detecting the correlation between testosterone and MRT only among men, we performed all subsequent regression analyses on MRT only for data from men.

The significant effect of pubertal testosterone on MRT in men remained when we entered testosterone and PDS (controlling for pubertal development) in the regression analysis predicting MRT performance ($F(2,90) = 4.25$, $p < .05$, partial $\eta^2 = .06$), while PDS was not a

significant predictor of MRT performance ($p = .69$, partial $\eta^2 = .002$). We performed further regression analysis with pubertal testosterone as a predictor of MRT performance among men, with OLM and Digit Symbol as covariates (controlling for other cognitive abilities due to their significant correlations with MRT). In this model, testosterone and Digit Symbol were significant predictors of MRT performance ($F(3,93) = 7.63$, $p < .001$). The effect sizes were partial $\eta^2 = .09$, and partial $\eta^2 = .08$ for testosterone measure and Digit Symbol, respectively. Adding PDS in this model did not change the results.

Within-family analyses of the association between pubertal testosterone and MRT among men

Next, we asked whether the individual-level association between pubertal testosterone and MRT performance in male twins as individuals would replicate in within-pair associations of twin brothers. In these analyses, we used continuous within pair difference scores in pubertal testosterone and within pair difference scores in MRT. In the individual-level analyses, the direction of correlation between pubertal testosterone and MRT was negative: male twins with higher age 14 testosterone tended to perform more poorly in MRT at ages 21–23. Accordingly, we evaluated significance of within-pair correlations with one-sided p -values, testing whether the twin brother with a higher level of pubertal testosterone was the twin brother scoring lower in MRT in early adulthood.

There was a significant negative correlation ($r = -.32$, one-sided $p = .03$, $n = 35$) between difference scores in pubertal testosterone and difference scores in MRT among all pairs of twin brothers. And similarly, for the 18 pairs of DZ twin brothers, differences in pubertal testosterone correlated negatively with differences in MRT performance ($r = -.42$, one-sided $p = .04$, Fig. 2C), but for the 17 pairs of MZ twin brothers, differences in pubertal testosterone were not significantly correlated with differences in MRT ($r = -.07$, one-sided $p = .39$, Fig. 2D). Among DZ twin brothers, the co-twin with higher testosterone performed less well on MRT in 12 out of 18 pairs: 67% of male DZ pairs showed the expected negative association, such that positive within pair differences in testosterone associated with negative within pair differences in MRT or negative within pair differences in testosterone associated with positive within pair differences in MRT, see panel C in Fig. 2).

In regression analyses (one-sided significance testing) with correction for non independence of clustered twin data, within pair differences in testosterone levels significantly predicted within pair difference in mental rotation performance among all 35 male pairs ($F(2,34) = 3.36$, $B = -3.17$, $p = .04$, partial $\eta^2 = .09$). This association was not significant after including within pair differences in PDS as a covariate ($F(2,32) = 1.35$, $B = -3.25$, $p = .06$, partial $\eta^2 = .08$), although the effect size was comparable to the model without PDS. Similarly, the within pair difference in testosterone levels was a significant predictor of within pair difference in MRT performance among 18 DZ male twin pairs ($F(2,17) = 3.82$, $B = -3.94$, $p = .03$, partial $\eta^2 = .18$). Here again, the association became non significant after including the within pair difference in PDS in the model ($F(2, 15) = 1.42$, $B = -5.14$, $p = .06$, partial $\eta^2 = .15$), but again, the effect size was comparable to the model without PDS.

Discussion

First, all observed sex differences in our sample of 308 twins were in expected directions, yielding a male advantage in mental rotation ability and female superiority in memory for object location and processing speed as represented by Digit Symbol performance. Age at neuropsychological testing was unrelated to performance in cognitive measures, a result reflecting the narrow age range at neuropsychological testing. Education had a significant effect on Digit Symbol and Digit Span, but not on MRT and OLM. This probably reflects the fact that Digit Symbol and Digit Span, both subtests from Wechsler scales, are more g-

loaded tests than the two spatial tests that measure specific cognitive domains relatively independent from general cognitive capacity.

Our main finding was a significant negative linear association between pubertal testosterone levels and MRT performance in adulthood among men. Our results suggests that the effects of testosterone in young adults may be limited specifically to spatial abilities with known male advantage. Depending on the model, pubertal testosterone explained six to eight percent of the variance in mental rotation performance in adulthood among men. This effect size is large when compared to the effect of education on Digit Span and Digit Symbol. Education explained nine percent of the variance in these tests. It is noteworthy that pubertal testosterone(measured 7 – 9 years earlier) and Digit Symbol performance (measured on the same day as MRT) explained equal amount of variation in mental rotation performance among young adult men; i.e. 8% and 7% of the variation in MRT was explained by testosterone and Digit Symbol, respectively (see Table 2 for correlations).

While we detected a negative association between testosterone levels and mental rotation ability among young adult males, several earlier studies reported that the association is positive (e.g. Silverman et al., 1999; Hooven et al., 2004). It should be noted that these earlier studies measured testosterone levels at the time of administration of mental rotation test in young adulthood, whereas our study measured testosterone levels in adolescence during pubertal development. It is possible that the testosterone influences cognition differently during development, although this conclusion cannot be drawn from our study. We note that many earlier studies of young adults have included only college/university students, while our results are from population-based sample. Our finding of negative association between testosterone and male favoring spatial ability among men does not stand alone. Similar findings have been reported earlier in young adult men, with mean age of 22 years (Moffat & Hampson, 1996) as well as in a sample of 35 to 80 year old men (Yonker et al., 2006).

That testosterone levels reflected pubertal development is a reasonable assumption, because our data clearly show that the more advanced stage of pubertal development was linearly related to higher testosterone levels among males. Given that assumption, our results can be interpreted as supporting earlier studies that have demonstrated the correlation between late pubertal maturation and good spatial abilities (e.g. Waber, 1976; Waber, 1977; Newcombe & Bandura, 1983). Rate of maturation had a negative, although non-significant, correlation with MRT performance among boys also in our study. However, it should be noted that the rate of pubertal development among boys, on average, was relatively immature. The mean PDS score of 2.07 in boys is only slightly above value 2, which indicates that pubertal development has only began. This has relevance when interpreting the effects of testosterone, as well: the levels of testosterone are not necessarily reflecting solely pubertal development in all boys, but can be also indicative of the levels of circulating testosterone in general (on the other hand, testosterone levels increased as a function of more advanced pubertal development among men).

Why did we not detect any association between testosterone and mental rotation performance in women? One possible reason is that the pubertal testosterone levels in women are much lower compared to those of men, resulting also in much smaller variation of testosterone levels among women. It is possible that much higher pubertal testosterone levels would be needed to result in long lasting effects on cognition. And estradiol, rather than testosterone, may play an important role in pubertal brain development in girls, as was suggested by the finding of the association between estradiol levels and gray matter structures among 10 – 15 year old girls but not in boys (Peper et al., 2009, see also Hausmann et al., 2000 on the effects of estrogen on spatial ability in women).

The sex specific organizational effects of testosterone on male favoring spatial abilities have been earlier suggested by the notion that men with CAH perform worse than non-CAH men and women with CAH perform better than non-CAH women in domain of male favoring spatial abilities (Puts et al., 2008). Possible prenatal effects of testosterone among women are suggested also by twin studies that have reported a significantly better mental rotation performance in women with a twin brother compared to women with a twin sister (Vuoksima et al., 2010) or non twin women with slightly older brother (Heil et al., 2011). Some support for the organizational effects of testosterone comes from a study of men with idiopathic hypogonadotrophic hypogonadism (IHH). Compared to controls, 19 men with IHH had impaired spatial abilities. (Hier and Crowley, 1982). In this study, five men with hypogonadotrophic hypogonadism acquired after puberty did not show impaired spatial cognition compared to controls. Further, androgen-replacement therapy among 6 IHH men did not affect their spatial cognition. The results of Hier and Crowley might suggest that androgen deficiency before or during, but not after, puberty may have long lasting effects on spatial cognition (i.e. impairment), although it should be noted that results from individuals with clinical conditions are not directly generalized to men in general. The results of our study suggest that among healthy men, higher testosterone levels are related to impaired spatial processing. Based on these observations there is a question on whether optimal levels of testosterone during pubertal developmental are needed to have good spatial abilities in the domain of male favoring spatial abilities.

Unfortunately, we cannot make any conclusion on whether the effect of testosterone in our study is organizational or activational in origin. Importantly, uncertainties remain due to the absence of information on lifetime exposure to testosterone and on current levels of testosterone, which are clear limitations of our study. The association between pubertal testosterone and mental rotation ability remained after controlling for physical pubertal development which might indicate that the effects of testosterone on spatial cognition are at least partly independent of pubertal physical maturation. Thus, it is possible that the testosterone levels were not reflecting only pubertal levels, but also, at least partly, activational testosterone levels in young adulthood. It is also possible that direct measure of testosterone was capturing the stage of pubertal development better than self-report measure among boys.

We emphasize the replicated association of pubertal testosterone and adult MRT performance obtained in confirmation of this relationship in a within-family design among male twin pairs. Regarding the within pair differences in testosterone and MRT performance, we observed the negative correlation only among dizygotic twin pairs, but not in monozygotic twin pairs, which suggests possible shared genetics effects between these two phenotypes (McGue et al, 2010). Because monozygotic twins are identical at the genomic sequence level, the non-existence of the correlation between testosterone and mental rotation ability in within pair analyses of monozygotic pairs indicates that some genetic covariance may explain variability in both phenotypes. Replicating the association in within-pair analyses of dizygotic twin brothers indicates that the unmeasured (third variable) family confounders do not account for the observed association between testosterone and mental rotation ability. The association of testosterone levels and MRT performance could represent effects of between-family variation in family structure and socio-economic status, parenting and household environments, etc. (see Dick et al, 2000). To assess this possibility, we performed within-family analyses of co-twins discordant for testosterone. Considering these within pair analyses, the correlations of within pair differences in testosterone and mental rotation ability among all male twin pairs and further among dizygotic twin pairs were even larger than in individual level between family correlation, but we note that these significance levels were based on one-sided p-values. Thus these results should be critically

evaluated. With larger sample sizes also bivariate genetic modeling would be an appropriate approach for studying the possible shared genetic effects between these two phenotypes.

In between-family analysis, our result remained after controlling for physical pubertal development. In contrast, the association between pubertal testosterone and mental rotation performance was not significant in within-family analyses when pubertal development was entered in the model. This might be a result of small sample sizes in these analyses, since the effect size of testosterone was not lower than in between-family analyses (also, it should be noted that the p-values fell just slightly outside the alpha level of 0.05). Thus entering a covariate that correlates highly with testosterone levels in these regression models with small sample sizes might not be an appropriate strategy. On the other hand, this might also reflect the fact that there is a strong genetic correlation between pubertal development and pubertal testosterone levels (Hoekstra et al., 2006).

To conclude, we have demonstrated that pubertal testosterone is related to spatial cognition after 7–9 years in early adulthood. This effect was found to be sex- and domain-specific, for it was evident only as a negative association between pubertal testosterone and adult mental rotation ability among men. Whether this association remains later in life is not known.

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Fig. 1. The effect of pubertal development on testosterone levels among males and females. CI = confidence intervals.

Fig. 2.

The relationship between testosterone and mental rotation test score among males (A, $r = -.27$) and females (B, $r = .02$). The relationship between within pair difference in testosterone and within pair difference in mental rotation test performance among 18 male dizygotic (C, $r = -.42$) and 17 male monozygotic (D, $r = -.07$) twin pairs.

Table 1

Means and sex differences in testosterone, pubertal development and cognitive measures. Effect sizes indicated by Cohen's *d* (positive values indicate higher mean in men).

	Men mean (SD)	Women mean (SD)	F	d
Testosterone (nmol/l)	0.13 (0.08)	0.06 (0.02)	66.14 ***	1.20
Pubertal Development S.	2.07 (0.47)	2.88 (0.55)	160.41 ***	-1.58
Mental Rotation Test	13.12 (4.85)	9.90 (4.27)	30.45 ***	0.70
Object Location Memory	13.80 (5.60)	15.33 (5.28)	5.00 *	-0.28
Digit Symbol	77.73 (13.89)	84.40 (13.83)	13.78 ***	-0.48
Digit Span	13.81 (3.05)	13.46 (2.72)	1.05	0.12

Note.

*
p < .05;

p < .001.

Mean testosterone levels based on two saliva samples are reported in nmol/l in this table, but we used adjusted log testosterone measure in all analyses.

Table 2

Correlations (N's in parentheses) among testosterone, pubertal development and cognitive measures in men (lower triangular) and in women (upper triangular).

	1.	2.	3.	4.	5.	6.
1. Testosterone		.09 (165)	.02 (176)	-.04 (173)	.05 (177)	-.02 (178)
2. Pubertal development	.57*** (124)		.03 (163)	.06 (160)	-.01 (164)	.10 (165)
3. Mental rotation test	-.27** (130)	-.12 (124)		.27*** (171)	.16* (175)	.17* (176)
4. Object location memory	-.01 (127)	-.01 (121)	.19* (127)		.21** (172)	.18* (173)
5. Digit symbol	-.10 (130)	.11 (124)	.31*** (130)	.03 (127)		.28*** (177)
6. Digit span	-.08 (130)	-.09 (124)	.11 (130)	-.01 (127)	.24** (130)	

Note.

*
p < .05;

**
p < .01;

p < .001.