



## Short communication

## Mental rotation in female fraternal twins: Evidence for intra-uterine hormone transfer?

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## ABSTRACT

Men outperform women in mental rotation by about one standard deviation. Prenatal exposure to testosterone has been suggested as one cause. In animals it has been shown that a female fetus located between two male ones is exposed to higher levels of testosterone. It is still unclear whether intra-uterine hormone transfer exists in humans. Therefore, the influence of an intra-uterine presence of a male co-twin was studied in female fraternal twins ( $N=200$ ). Women with a male co-twin outperformed women with a female co-twin by about a third standard deviation. In a no-twin control group ( $N=200$ ), performance of women with a slightly older sibling did not depend upon the sibling's sex. These findings provide preliminary support for the theory of an influence of prenatal testosterone on mental rotation performance.

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## 1. Introduction

The process of imagining the representation of an object turning around is called mental rotation (Shepard and Metzler, 1971) and constitutes an important aspect of spatial cognition and intelligence. An instrument widely used to assess this ability is the mental rotation test (MRT; Vandenberg and Kuse, 1978; Peters et al., 1995), involving the comparison of 3D block figures. Typically, in the MRT men score about one standard deviation higher than women (Voyer et al., 1995). Although both biological as well as psychosocial suggestions have been put forward to explain this sex difference, discussion will focus on the former ones because our research tested a specific biological hypothesis.

Hemispheric lateralization of brain activity during mental rotation as a function of sex was reported rather consistently (see, e.g., Vogel et al., 2003), with a larger right parietal activity observed in men and a (at least relatively) larger left parietal activity in women. Sex hormones have organizational effects on brain structure and function and also have subsequent activational effects on the brain. The latter has repeatedly been demonstrated by the observation that the continuous steroid fluctuations in normally cycling women lead to concomitant changes of sex differences

both in performance and in cerebral asymmetries (Hausmann, 2005). Evidence for the organizational effects, however, is less conclusive (Güntürkün and Hausmann, 2003), at least in humans. Indirect evidence comes from the observation that the brain effects described above require neither the hormonal changes related to puberty itself nor the post-pubertal steroid fluctuations to occur. Hahn et al. (2010a, b) provided evidence for sex differences in functional cerebral asymmetry during mental rotation in preschool children. Moreover, Moore and Johnson (2008) and Quinn and Liben (2008) demonstrated that behavioral sex differences in mental rotation already exist in infants, thus pointing towards a very early causation (and thus also against experience-related accounts). Unfortunately, methods to study the effects of prenatal testosterone exposure in humans suffer from certain problems and also yielded inconsistent results (Cohen-Bendahan et al., 2005).

The comparison of female twins with a same-sex (SS) versus an opposite-sex (OS) fraternal co-twin could offer evidence on the effects of prenatal testosterone exposure (Miller, 1994). Intra-uterine hormonal transfer effects have been demonstrated in rodents and other litter-bearing species (Ryan and Vandenberg, 2002), but whether such effects occur in humans remains in dispute. There have been reports of positive findings for, e.g., tooth size (Dempsey et al., 1999), for spontaneous otoacoustic emissions (McFadden, 1993), or for cerebral lateralization assessed with a verbal dichotic listening task (Cohen-Bendahan et al., 2004), but there are also negative findings for, e.g., handedness (Elkadi et al., 1999;

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Medland et al., 2009), for digit ratio (Medland et al., 2008), or for gender-typed toy play (Rogers et al., 1998).

Early in pregnancy, direct hormone transfer from one twin to the other may exist as steroids cross the placenta, the fetal membranes and the fetal skin (Abramovitch and Page, 1972). Later in pregnancy, changes in the fetal skin prevent simple diffusion, but hormones from one twin may reach the other via trans-membrane transport and the maternal–fetal circulation (Meulenberg and Hofman, 1991). Little is known about the effectiveness of these mechanisms and the brain's sensitivity to testosterone during different periods of pregnancy with respect to spatial cognition.

Nevertheless, based on the hypothesis that the testosterone of a male member of an OS pair may influence the female co-twin, and that prenatal testosterone exposure has long-term effects on brain structure and function relevant for spatial cognition, we expect that female twins with a male co-twin should show higher mental rotation performance than female twins with a fraternal female co-twin, as very recently found by Vuoksima et al. (2010). In addition to the twin sample, non-twin control females participated in our study with either an older brother or an older sister close in age, i.e., with an age difference of not more than 18 months. If having being raised with either a sister or a brother might be crucial for mental rotation performance instead of prenatal hormone transfer, then similar effects should be found for the twin as well as for the non-twin samples.

## 2. Experiment 1

### 2.1. Methods

#### 2.1.1. Participants

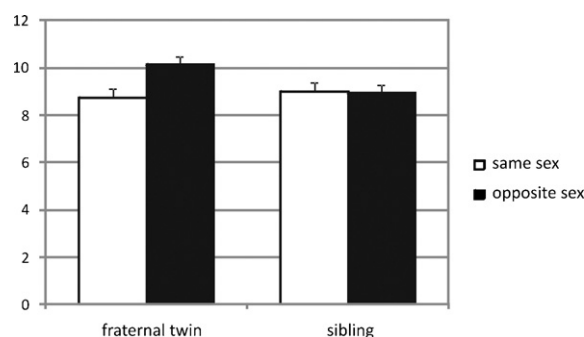
In this study, 400 women aged 19 until 39 years with a high school degree participated. A variety of recruitment methods were used, including undergraduate class announcements, e-mail listserve advertisements, and university newspapers. Participants were recruited from about 10 universities all over Germany. The fraternal twin sample included 200 women (mean age: 23.6 years), with 100 from a same sex dizygotic twin pair and 100 with an opposite sex twin. Zygosity of the SS twin pair was based on self-reports. The non-twin controls (mean age: 23.3 years) included 100 women with an older brother close in age (age difference ranging between 9 and 18 months, mean age difference: 14.4 months) with whom they were raised and 100 women with a 9–18 months older sister (mean: 15.6 months).

#### 2.1.2. Procedure

Potentially interested participants contacted the first author, and were informed about the study procedures. 97% were interested in receiving the MRT questionnaire. They were instructed that they needed someone controlling the time when solving the MRT, and that they strictly had to meet the requirements (see below). The questionnaire had to be returned with some additional demographic information, and the return rate amounted to 94%.

Participants had to solve the MRT in its version A (Peters et al., 1995). The MRT is made of three-dimensional cube figures in two sets with 12 items each. Six items were presented per sheet of paper. Each item contains a target on the left side and four sample stimuli on the right. Two stimuli were identical but in-depth rotated versions of the target. The two remaining stimuli did not match regardless of rotation. The participants were asked to cross out both correct sample stimuli. Participants were given 3 min for the first 12 items and after 4 min break another 3 min for the remaining 12.

The standard scoring method (Peters et al., 1995) was used, i.e., one point was given if both and only both correct sample stimuli were marked correctly. A maximum of 24 points could thus be



**Fig. 1.** Mental rotation performance (MRT score) of Experiment 1 as a function of "relation" (fraternal twin vs. regular sibling) and "sibling's sex" (same vs. opposite). Error bars indicate standard errors.

obtained.

Given a sample size of  $N=400$  and  $\alpha=.05$ , a greater "sibling's sex" effect for fraternal twins compared to regular siblings of size  $d=0.3$  (see Vuoksima et al., 2010) could thus be detected with a probability of  $1 - \beta = .91$ .

### 2.2. Results and discussion

A two-way analysis of variance with the between-subject factors "relation" (fraternal twin vs. regular sibling) and "sibling's sex" (female vs. male) revealed no main effect of relation ( $F(1, 396)=1.57, p>.20$ ) and a trend of sibling's sex ( $F(1, 396)=3.01, p=.083$ ) on MRT performance. Most importantly, however, a significant interaction was obtained ( $F(1, 396)=3.90, p<.05$ ), see Fig. 1. For non-twin controls, MRT performance did not differ as a function of sibling's sex ( $F(1, 198)=0.03$ ). Women with an opposite sex twin, however, scored significantly higher in the MRT than women with a same sex twin ( $M=10.12$  vs.  $8.73, SD=3.65$  vs.  $3.68; F(1, 198)=7.20, p<.01$ ). The effect size of this difference amounted to  $d=0.38$  (95% confidence interval: 0.10–0.66). Additionally, opposite sex twins scored higher than non-twin controls who were raised with a slightly older brother ( $F(1, 198)=5.37, p<.05$ ), see Table 1.

Our data were fully consistent with the hypothesis that increased prenatal testosterone exposure in females masculinizes mental rotation performance: OS female twins had a higher mental rotation performance than SS female twins. This main effect is the most important result and the best test of the testosterone exposure theory since the twin samples offer the perfect age- and environmental-matched design. Furthermore, OS female twins also had higher mental rotation performance than non-twin females who were raised with an older brother. These findings highlight the possibility of organizational effects of prenatal testosterone.

**Table 1**

Mean MRT score (with standard deviation in parentheses) and sample sizes of Experiment 1 as a function of "relation" (fraternal twin vs. regular sibling) and "sibling's sex" (same versus opposite).

	Sibling's sex		
	Brother	Sister	Mean
Relation			
Fraternal twins	10.12 (3.65) $N=100$	8.73 (3.68) $N=100$	9.43 (3.72) $N=200$
Regular siblings	8.91 (3.74) $N=100$	9.00 (3.92) $N=100$	8.95 (3.82) $N=200$
Mean	9.52 (3.73) $N=200$	8.87 (3.79) $N=200$	9.19 (3.77) $N=400$

**Table 2**  
Mean MRT score (with standard deviation in parentheses) of Experiment 2 as a function of group and testing session and the correlation between the two sessions.

	1st testing	2nd testing	Correlation
Group			
Self/experimenter administered $N = 75$	8.87 (3.81)	9.01 (3.90)	.720
Experimenter-/experimenter administered $N = 75$	8.81 (3.89)	8.98 (3.91)	.695

### 3. Experiment 2

One might argue, however, that administering the MRT by post does not guarantee reliable and valid measures of mental rotation performance, although it enables a larger sample size than testing in the lab with a trained researcher.<sup>1</sup> Thus, we conducted a control experiment with two groups of females who solved the MRT twice. The first testing was self-administered at home for one group but administered by a trained researcher in our lab for the second group. Two weeks later, both groups solved the MRT administered by a trained researcher in our lab.

#### 3.1. Methods

##### 3.1.1. Participants

In this control study, 150 women aged 19 until 39 years (mean age: 22.9 years) with a high school degree were recruited from 2 universities. They were informed that there were two sessions with 2 weeks in between (mean: 14.6 days) but did not expect to solve the MRT again during the second session.

##### 3.1.2. Procedure

Seventy-five women were informed about the study procedures to be followed at home in exactly the same way we used in Experiment 1 (self-administered group). The MRT had to be returned by mail with some additional demographic information. In the researcher-administered conditions (second testing for the just-mentioned group plus both testing sessions for the remaining 75 participants) participants were tested individually in our lab by an experienced researcher.

#### 3.2. Results and discussion

A two-way analysis of variance with the between-subject factors “group” and “session” (1st vs. 2nd testing) revealed no main effect and no interaction (all  $F$ -values  $< 1.25$ , all  $p > .25$ ), see Table 2. The correlation between the first and the second testing sessions were high, and did not differ as a function of group. Thus, self-administration of the MRT does not pose a problem for the reliability or the validity of this score.

### 4. General discussion

Clear sex differences in mental rotation exist, in which males outperform females by about one standard deviation (e.g., *Voyer et al., 1995*; but see *Jansen-Osmann and Heil, 2007*). Prenatal testosterone exposure was suggested as the cause for this sex difference (*Cohen-Bendahan et al., 2005*) that at a behavioral level was already observed in infants (*Moore and Johnson, 2008*; *Quinn and Liben, 2008*) and at the level of sex-specific lateralization recently was

observed in preschool children (*Hahn et al., 2010a,b*). During prenatal development, testosterone masculinizes the developing central nervous system (CNS) in males (*Becker et al., 2005*). Animal studies confirm that testosterone’s masculinization of the CNS translates into several sexually dimorphic behaviors, including spatial cognition (*Hampson, 1995*).

To our knowledge, this is the second fully published study (*Vuoksima et al., 2010*; see also *Cole-Harding et al., 1988*) to use OS and SS female twins to explore the effects of prenatal testosterone exposure on mental rotation performance. Overall, our data were fully consistent with the hypothesis that increased prenatal testosterone exposure in females masculinizes mental rotation performance: OS female twins had a higher mental rotation performance than SS female twins. Our effect size ( $d = 0.38$ ) indeed was very similar to the  $d = 0.30$  reported by *Vuoksima et al. (2010)*. Furthermore, we demonstrated that OS female twins also had higher mental rotation performance than non-twin females who were raised with an older brother (there was no such control group in *Vuoksima et al., 2010*). Admittedly, there may be something unique about having a brother the exact same age so that a psychosocial explanation cannot completely be ruled out. Nevertheless, our findings highlight the possibility of organizational effects of prenatal testosterone.

Although Experiment 2 suggested that self-administration of the MRT does not pose a problem, some limitations must be noted. First, only academics participated, thus the extent to which our results are representative of the general population remains to be tested. Second, zygosity status was self-reported and information on placenta type (i.e., monochorionic vs. dichorionic, probably affecting the level of intra-uterine hormone transfer) was not available. According to *Vuoksima et al. (2010)*, however, MRT performance of SS female fraternal twins did not differ from female monozygotic twins’ performance. Finally, menstrual cycle status was not controlled in the present study.

Clearly, the possible elevated prenatal testosterone exposure of OS twin females, the extent of such an effect, and also its influence on spatial cognition remains to be investigated in more detail. At the present, our findings are suggestive of a prenatal testosterone effect on adults’ mental rotation performance. Testosterone binds to the X chromosome linked androgen receptor, which contains a polymorphic polyglutamine CAG repeat. The length of this CAG repeat is positively correlated with testosterone levels in males, and negatively correlated in females (for a review, see, e.g., *Medland et al., 2005*). Thus, it would be interesting whether or not the number of CAG repeats can predict mental rotation performance.

Finally, we strongly suggest the following 2 studies for the near future that should provide more information about the role of prenatal testosterone. First, the study of *Moore and Johnson (2008)*; see also *Quinn and Liben (2008)* that demonstrated behavioral sex differences in mental rotation already in infants should be replicated with female fraternal twins. Effects of the co-twin’s sex obtained at an early age in the first year of life would strongly suggest a causal role of prenatal testosterone instead of a psychosocial explanation based on a shared social environment with an opposite sex co-twin. Second, the present study should be replicated while measuring brain activation to investigate whether OS female twins show a masculinized pattern of hemispheric lateralization (e.g., *Yu et al., 2009*).

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<sup>1</sup> Form the method section of *Vuoksima et al. (2010)*, it is not clear whether or not in their study the MRT was administered to about 800 twins by an experimenter or was self-administered.

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