Missing evidence for the effect of one-week phytoestrogen-rich diet on mental rotation in two dimensions

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Abstract

INTRODUCTION: It is well known that men outperform women in tests of spatial cognition, such as mental rotation, and that performance in these tasks is influenced by sexual hormones. Phytoestrogens are plant substances chemically similar to estradiol, capable of binding estrogen receptors. In a previous study, one-week phytoestrogen-rich diet improved performance in mental rotation in women, but it remained unclear whether this was due to direct binding of phytoestrogens to estrogen receptors or by modulating testosterone blood levels.

OBJECTIVES: We investigated whether one week consumption of phytoestrogens will equally affect performance in mental rotation in women and men. We expected improved performance in women, but unchanged skill in men, if the effects were mediated by changes of testosterone level. On the other hand, direct modulation of estrogen receptors should yield comparable improvement in both genders.

METHODS: Thirty-six healthy adult young volunteers (16 females) were divided into the control and soy groups. During 7 consecutive days, in addition to their usual diet, subjects in the soy group consumed a daily dose of 2 g of soybeans per kilogram body weight (about 170 mg/kg of isoflavones). The control group members stayed on their usual diet. The subjects were tested on mental rotation of letters and digits at the baseline and after 7 days of soy consumption.

RESULTS: Consumption of soy had no influence on performance in the mental rotation task. For the intermediate stimulus rotation angle (60 deg), irrespective of soy intake, improvement of response latencies was greater in women than in men.

CONCLUSION: The results indicate that soy phytoestrogens have little impact on mental rotation in two dimensions. The findings also suggest that women, in contrast to men, more readily engage memory mechanisms to solve the mental rotation task.

ORIGINAL ARTICLE

INTRODUCTION

It is well known that gender differences exist in cognitive functions (Linn and Petersen, 1985; Hampson and Kimura, 1992). It was repeatedly confirmed that spatial cognition (mental rotation, spatial visualization) and mathematical/logical reasoning are better developed in men; whereas women outperform men in verbal abilities, visual memory and perceptual speed (Maccoby and Jacklin, 1974; Kimura, 2002). The basic principles of these differences are intensively discussed. Genetic influence, speed of central nervous system maturation, and hormonal effects are regarded as possible factors (McGee, 1982; Sanders and Soares, 1986; Voyer and Bryden, 1990). It has been reported that prenatal levels of sex steroids affect brain growth, maturation, brain structures organization and development of functional brain asymmetry (Geschwind and Galaburda, 1985; Hier and Crowley, 1982; Christiansen and Knussman, 1987; Hampson and Kimura, 1992; Kimura 2002; Ostatnikova *et al*., 2002).

Fluctuations of sex steroids levels in adult organism have been associated with variations in cognitive functioning as well. In mental rotation, in women, better performance has been related to higher levels of testosterone and lower levels of estradiol (Hampson and Kimura, 1992; Hausmann *et al*., 2000; Komnenich *et al*., 1978). In men, on the other hand, certain levels of testosterone seem to be optimal for the performance so that deviations in both directions from the optimal level affect the performance negatively (Celec *et al*., 2002; Choi and Silverman, 2002; Gouchie and Kimura, 1991).

The effect of testosterone on nerve cells is indirect, via its metabolite estradiol (Garcia-Segura *et al*., 2003). After binding specific estrogen receptor (ER), estradiol affects cell metabolism by changes of ions concentration, membrane excitability, and other mechanisms (Benten *et al*., 2004). Two forms of ER exist, ER-α and ER-β (Kuiper at al., 1997), which have different distribution and function in the brain (Osterlund at al., 1998; Nilsson and Gustafsson, 2002). ER-β are distributed mostly within the hippocampus and the frontal cortex and have been related to cognitive processing (Kuiper *et al*., 1998). ERs also bind substances structurally similar to estradiol including phytoestrogens, natural substances found in high concentrations in beans, particularly soy (Adlercreutz *et al*., 1993; Davis at al., 1999).

Numerous studies have reported positive effect of phytoestrogens, especially isoflavones, in prevention of several civilization diseases. For this reason phytoestrogens are currently a hotly debated topic (Cornwell *et al*., 2004; Glazier and Bowman, 2001, Knight and Eden, 1996; Karvaj *et al*., 2007). A much discussed issue is possible use of phytoestrogens as alternative hormonal substitution therapy for women in menopause (Beck *et al*., 2005; Glazier and Bowman, 2001) and in prevention of hormone dependent cancer (Kurzer, 2000). It has been shown that high doses of phytoestrogens have positive influence on cognitive functions, especially memory and learning (File at al., 2001; Duffy at al., 2003; but see Kreijkamp-Kaspers *et al*., 2007). Ostatnikova and co-workers reported that a one-week high-dose phytoestrogen diet improved performance in mental rotation in women (Ostatnikova *et al*., 2003; Celec at al., 2005).

The mechanism of effect of phytoestrogens on cognitive functions, including mental rotation, remains poorly understood. Some authors explain this influence as dependent of changes in hormonal level, testosterone in particular. Consumption of high doses of phytoestrogens may increase blood concentration of testosterone (Celec *et al*., 2005; Tarrago-Castelanos, 2006). Lund and co-workers (Lund *et al*., 2001) reported that female rats fed with high doses of phytoestrogens improved in solving a maze task. In contrast, performance of male rats on such diet was impaired. The authors proposed that performance might depend on the ratio of testosterone to estradiol. Phytoestrogens might influence cognitive processing also by direct modulation of ERs. Phytoestrogens are partial agonists of ERs, in particular ER-β (Barnes *et al*., 2000, Belcher and Zsarnovszky, 2001; Wollmer *et al*., 2002). It has been suggested that this mechanism could mediate the effect of phytoestrogens on learning and memory (Lund and Lephard, 2001; Lephard *et al*., 2004).

The aim of this study was to examine whether a one-week high-phytoestrogen diet, which was found to influence testosterone levels in previous studies (Celec *et al*., 2005; Ostatnikova *et al*., 2007), would equally affect mental rotation performance in women and men. As it was mentioned above, the effect of phytoestrogens on cognitive functions could be mediated by two mechanisms. If the effect of phytoestrogens on mental rotation were primarily determined by testosterone level changes, we might expect that performance of women will improve but performance of men will not change or will even get worse. On the other hand, if the effect of phytoestrogens were mostly mediated by ER modulation, affecting learning and memory, we could expect rather comparable performance improvement in both sexes.

MATERIAL AND METHODS

Subjects

Thirty-six healthy right-handed volunteers (16 females, mean age 24.6 ± 2.2) participated in the study. They were recruited among undergraduate and graduate university students. Informed consent was required and the study was approved by the local ethical committee. A soy group included 7 females and 10 males; a control group included of 9 females and 10 males. The subjects had no history of endocrinological, neurological or psychiatric disorders. No subject was using endocrine substitution or contraception. The subjects were asked not to ingest any additional soy-derived supplements, to

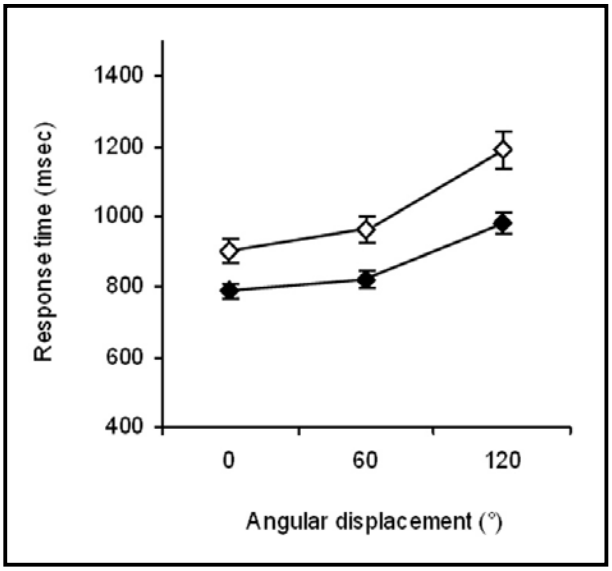

Fig. 1. Mean response time (RT) in mental rotation of alphanumeric characters before (filled symbols) and after 1 week (empty symbols). Errors bars indicate standard error of the mean.

Tab. 1. Results of the RM-ANOVA showing all factors and interactions. Values of Greenhouse-Geisser epsilon are shown in case Mauchley's test of sphericity was significant (indicating sphericity violation). Significant effects are indicated by asterisk.

Factor	F	df	ε	p
Angle	100.105	2, 64	0.596	0.000*
Angle × Sex	0.009	2, 64	0.596	0.952
Angle × Soya	3.178	2, 64	0.596	0.076
Angle × Sex × Soya	0.172	2, 64	0.596	0.752
Time	36.031	1, 32		0.000*
Time × Sex×	0.747	1, 32		0.394
Time × Soya	0.111	1, 32		0.741
Time × Sex × Soya	0.507	1, 32		0.482
Time × Angle	8.857	2, 64	0.688	0.002*
Time × Angle × Sex	0.633	2, 64	0.688	0.479
Time × Angle × Soya	1.577	2, 64	0.688	0.22
Time × Angle × Sex × Soya	0.247	2, 64	0.688	0.698
Sex	0.150	1, 32		0.701
Soya	0.941	1, 32		0.339
Sex × Soya	1.665	1, 32		0.206

prevent excessive physical activities, and abstain from drinking alcohol. In addition to usual food, the soy group was ordered to eat soybeans in amount of 2 g/kg/day during 7 consecutive days. No restriction concerning the soy meals preparation was given. The dose of 2 g/kg/day was chosen for the following reasons. Unprocessed soybeans contain 1.2–4.2 mg of isoflavones per 1 g of dry weight (Wang and Murphy, 1994). Considering the lower estimate of isoflavones concentration in soybeans, the daily intake was about 170 mg/kg/day, which exceeds the average supply of isoflavones from usual Asian diet (20–150 mg). Importantly, this amount of soy intake has been found to affect hormonal status in previous studies (Celec *et al*., 2005; Ostatníková *et al*. 2007).

Mental rotation test

Alphanumeric characters (P, R, S, F, Q, 1, 2, 4, 5 and 7) extending 2x1 cm were presented in the center of a computer monitor. The stimuli were rotated clockwise by 0° (no rotation), 60° or 120° and were displayed in regular or mirror-reversed form. Stimuli were presented in random order with total of 180 (3 × 60) trials per subject. Subjects were seated in a comfortable armchair; the distance to the monitor was 100 cm. By pressing keys, subjects indicated whether the stimulus was presented in regular or in mirror-reversed form (2-alternative forced choice). Subjects were instructed to respond with high accuracy while to be as fast as possible. Judgment and response time were registered. A series of practice trials was administered before the experiment. The experiment continued only in case the subject delivered at least 95% of correct answers in 20 consecutive trials. Short breaks in stimuli presentation were introduced to prevent exhausting subjects. The subjects were tested twice, at the baseline and after 7 days.

Data analysis

The paradigm used in this experiment typically yields high response accuracy (Cooper and Shepard, 1973; see Results) so that response time (RT) is the only valid performance index. In order to analyze response latencies error trials were discarded along with trials with RT shorter than 300 ms and longer than 3000 ms. Response time data were analyzed using a 4-way repeated measures analysis of variance (RM ANOVA) with between-subject factors Soy, Sex and within subject factors Angle (0°, 60°, 120°) and Time (baseline, 7 days after). An additional within-subject factor Form (regular, mirror) was included in further analysis. Greenhouse-Geisser correction for sphericity violation was applied when appropriate.

RESULTS

Response time (RT) increased with increasing angular displacement of the stimulus (Fig. 1, Table 1, Angle: $p < 0.001$). This was observed in all subjects. Response accuracy was high for all angular displacements (0° = 98.4%, 60° = 98.3%, 120° = 95.4%). Therefore, RT provided the only reliable index of test performance, which is usual in experiments employing alphanumeric characters (Cooper and Shepard, 1973; Heil *et al*., 1998).

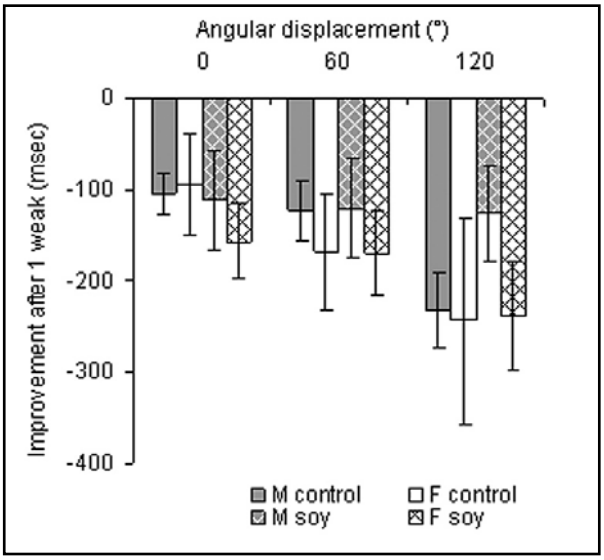

Fig. 2. Improvement in mental rotation performance after 1 week (RT on repeated testing – RT at the baseline). Data are shown for the soy group and the controls in females and males. Errors bars indicate standard error of the mean.

On the repeated examination, 7 days after the baseline measurement, RTs were shorter in all subjects. The RT improvement was more pronounced for greater stimulus angular displacement (Fig. 2, Table 1, Time x Angle interaction: $p = 0.002$). Neither gender nor soy consumption influenced the mental rotation performance (Table 1).

We considered the possibility that missing effects of soy consumption could result from rather low task difficulty. It is well known that mental rotation of mirror-reversed symbols requires longer time than mental rotation of regularly displayed characters (Windes, 1991). Because of this in the next step we included the form of character presentation (regular versus mirror-reversed) as an additional factor. RT for the mirror-reversed stimuli was significantly longer than RT for characters that were presented in their canonical form (Form: $F = 70.962$; $df = 1, 32$; $p < 0.001$). The increase in RT with increasing stimulus rotation was greater for the mirror-reversed characters than for the regularly presented stimuli (Angle × Form: $F = 14.162$; $df = 2, 64$; $p < 0.001$). By repeated test the improvement in RT was greater for the mirror-reversed characters than for the regularly displayed stimuli (Time x Form: $F = 4.438$; $df = 1, 32$; $p = 0.043$). The effects of gender and soy consumption on RT were significant neither for the mirror-reversed characters nor for the regularly displayed stimuli.

Next we evaluated selectively the data for characters "2" and "S". These stimuli yielded the longest RTs so that the mental rotation of these characters was considered the most difficult. This was also confirmed by subjective assessments of study participants. For these selected stimuli the effects of factors Angle and Time were significant too (Table 2). The improvement of RT was more pronounced for greater stimulus rotation, but it was different in men and women. As shown in Fig. 3, for stimuli rotated by 60° the improvement was greater in females than in males (Time × Angle x Sex: $p = 0.042$). Even in this case, however, no effect of soy consumption was observed.

Tab. 2. Results of the RM-ANOVA for the "demanding characters" ('2' and 'S'). Mauchley's test of sphericity was insignificant for all factors/interactions investigated. Significant effects are indicated by asterisk.

Factor	F	df	p
Angle	71.256	2, 64	0.000*
Angle × Sex	0.291	2, 64	0.744
Angle × Soya	1.896	2, 64	0.160
Angle × Sex × Soya	0.131	2, 64	0.872
Time	35.035	1, 32	0.000*
Time × Sex	1.005	1, 32	0.324
Time × Soya	0.037	1, 32	0.850
Time × Sex × Soya	0.354	1, 32	0.556
Time × Angle	7.082	2, 64	0.002*
Time × Angle × Sex	3.337	2, 64	0.042*
Time × Angle × Soya	1.822	2, 64	0.17
Time × Angle × Sex × Soya	0.248	2, 64	0.779
Sex	0.071	1, 32	0.792
Soya	0.615	1, 32	0.439
Sex × Soya	1.514	1, 32	0.228

DISCUSSION

The aim of this study was to investigate the impact of high doses of dietary soy phytoestrogens on sex differences in mental rotation performance. Previous studies have pointed to the influence of phytoestrogens on cognitive functions. It is not clear currently, whether such effects are mediated by change of serum testosterone level, or direct influence on ER-β. In the first case, we expected performance improvement in women only. In the second case, we expected similar improvement in both sexes. In fact, we did not confirm the effect of phytoestrogens on performance in mental rotation in any sex. Although repeated testing 7 day after the baseline measurement revealed different performance improvement in men and women, this difference was unrelated to soy consumption. We note that due to high response accuracy RT is the only reliable index of task performance in this paradigm (Cooper and Shepard, 1973; Heil *et al.*, 1998), which was confirmed by our measurements too.

In our earlier studies, restricted to females, we found that a one-week consumption of high doses of soybeans phytoestrogens improved performance in mental rotation (Celec *et al*., 2005; Ostatnikova *et al*., 2002). These findings were not confirmed in the current study. After one week the time needed to solve the task was shorter, but this improvement was independent of soy intake (in both sexes). There are several possibilities how to explain the missing effect of phytoestrogens on performance in the mental rotation task.

First, the type of stimuli may be mentioned. In the present study we used alphanumeric characters rotated in two dimensions in the frontal plane. In contrast, in our earlier studies mental rotation of objects in three dimensions was used. The latter task is more demanding, which is reflected by longer RT. For this reason, we selected the characters yielding the longest RTs, i.e. we selectively evaluated the most difficult condition of our experimental setup. However, even in this condition, we did not register any effect of soybeans intake. It is possible that even the most difficult condition of two-dimensional mental rotation of characters does not equal the difficulty of mental rotation in three dimensions. The absence of sex differences in the baseline measurement might be attributed to low task difficulty too. It has been suggested that sex differences in mental rotation are more clearly found with difficult paradigms (Collins and Kimura, 1997; Jansen-Osman and Heil, 2007).

Second reason for the missing effects of soy intake might be a low dose and/or short duration of soybeans consumption. However, with this respect the present study does not differ from our earlier studies, which proved effects on hormonal levels and improvement in mental rotation in females (Celec *et al*., 2005; Ostatnikova *et al*., 2002, 2007). It is possible, however, that higher doses of isoflavones and longer intake are needed in order to influence performance in less difficult tasks (File *et al*, 2001).

Finally, our results suggest that the effect of soy consumption on performance in mental rotation is low, if any. Therefore, larger sample could be needed to disclose such small effect size.

The analysis of mental rotation performance on repeated testing revealed an interesting sex difference. For the "demanding characters" ('2' and 'S') rotated by 60° we found significantly greater improvement of RT in females than in males. This difference might be attributed to different cognitive strategies used by women and men. Heil *et al.* (1998) showed that performance (i.e. RT) improvement on repeated mental rotation tests may be primarily due to establishing new explicit memory representations of rotated stimuli rather than enhanced efficiency of the imaginary visual-spatial object manipulation. In solving cognitive tasks, women use memory strategies more readily than men (Levy *et al*., 2005). By small angles of rotation the stimuli are closer to the stored memory representation of characters so that rather easy memory encoding is expected. Our results thus indicate that, for small rotation angles, women, as opposed to men, preferentially engage memory strategies when they repeatedly solve the mental rotation task. A greater rotation angle (such as 120˚) might require full engagement of visual-spatial transformation (i.e. „mental rotation" in the strict sense of the word) in both sexes. Similarly, Vingerhoets *et al.* (2001) have shown that subjects invariably use „proper" mental rotation in difficult tasks, while they choose from several possible strategies in simpler tasks. The individual choice of particular strategy depends not only on task difficulty but also on the level of development of spatial abilities (Kosslyn *et al*., 1998).

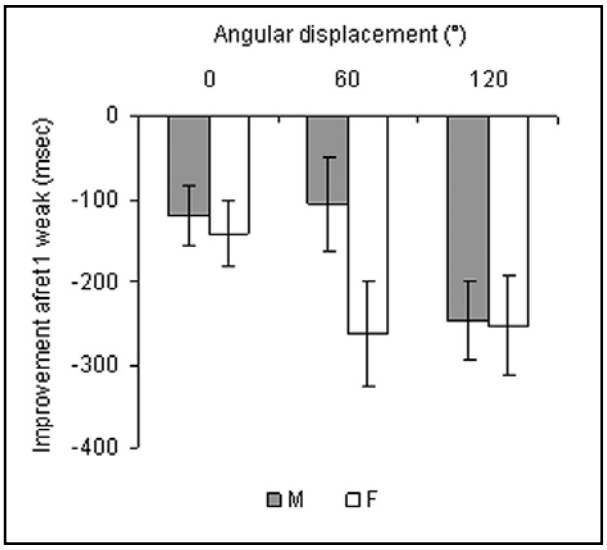

Fig. 3. Improvement in mental rotation performance after 1 week for the "demanding characters" ('2' and 'S') in females and males (irrespective of soy intake). Errors bars indicate standard error of the mean.

The aim of this study was to assess whether short-term diet with high concentration of phytoestrogens may differently affect performance in mental rotation in women and men. We did not find different effect of soy consumption in women and men. Therefore, we can make no claims about the mechanisms, by which phytoestrogens could improve mental rotation performance. Our results suggest that in order to be detected the effect of short-time high phytoestrogen intake might require large samples or more demanding mental rotation paradigms.

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