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Effect of daidzein on anxiety, social behavior and spatial learning in male Balb/cJ mice

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ABSTRACT

Daidzein is an abundant isoflavone present in soy. It is unique as it can be further metabolized into equol, a compound with greater estrogenic activity than other isoflavones. The potential benefit of daidzein in the prevention of various cancers and cardiovascular diseases has drawn attention to this molecule and isoflavone consumption is increasing around the world. However, it remains unclear whether daidzein affects locomotor activity, anxiety, social behavior or spatial memory. Here we report the results from a range of tests designed to assess anxiety, social behavior and spatial learning and memory in male Balb/cJ mice following consumption of daidzein for 30 days. We found that daidzein treatment significantly increased locomotor activity in the open field, elevated plus-maze and Morris water-maze tests; resulted in increased amicable behavior, decreased aggression and decreased sexual behavior during social interaction tests; and resulted in an anxiolytic effect amongst treated males. We found no effect of daidzein consumption on the acquisition and retrieval of spatial memory. Our results suggest that long-term consumption of daidzein may produce significant effects on locomotor activity, mood and social behavior without significant effects on learning and memory.

1. Introduction

Phytoestrogens are plant compounds similar to estradiol in structure and function that have the ability to selectively bind to estrogen receptors (ERs) (Kuiper et al., 1998). Of all phytoestrogens, soy-derived isoflavones are the most abundant in rodent and human diets and extensively studied in both animal and clinical research (Lephart et al., 2002). Phytoestrogens have protective effects against hormone-dependent cancers, cardiovascular diseases, osteoporosis, and the symptoms of menopause (Lephart et al., 2002). Genistein and daidzein are specific isoflavones found in soy-based diets and can exert estrogen-like effects on behavior. For example, soy is known to reduce anxiety in rats during an elevated plus-maze test (Lund and Lephart, 2001) and pubertal exposure to total isoflavones in adult male Syrian hamsters (*Mesocricetus auratus*) alters adult plasma hormone levels and affects aggressive behavior (Moore et al., 2004). Extended consumption of a high total isoflavone diet over a lifetime has been shown to negatively affect performance in a radial arm maze in male rats, but females fed isoflavone performed better than females fed isoflavone-free diets (Lund et al., 2001). Neonatal treatment with genistein and daidzein can result in defeminization of some traits such as suppressed receptivity (e.g. lordosis behavior) and reduced ovaries in female adult rats (Kouki et al., 2003). In addition, soy phytoestrogen dietary during adulthood significantly diminished lordosis behavior of female rats (Patisaul et al., 2004). Research in this field suggests that isoflavones can have profound effects on behaviors such as anxiety and aggression, visual–spatial memory, and reproductive behavior in adult animals.

Daidzein is a major component of isoflavones present in soybeans and other legumes. Although the effects of total isoflavone or genistein on animal behavior have been thoroughly investigated and the effects of daidzein, including its potential benefits, have also been investigated (Fonseca and Ward, 2004; Zhao et al., 2004; Lo et al., 2007; Huang et al., 2008), it remains unclear whether daidzein has any affect on anxiety, social behavior, or spatial learning. While daidzein has a low affinity to both the α and β types of estrogen receptors (ERα and ERβ) (Kuiper et al., 1998), it can be further metabolized into equol (Rowland et al., 2000; Setchell et al., 2002): a chiral molecule existing as the enantiomers R-equol and S-equol (Muthyala et al., 2004). S-equol exhibits a similar binding capacity to ERβ as genisten whereas R-equol shows a weak binding capacity to ERα (Latonnelle et al., 2002; Muthyala et al., 2004; Setchell et al., 2005). While mice, rats and monkeys consistently produce high levels of equol under normal feeding scenarios (Muthyala et al., 2004), mice can produce elevated amounts of equol when given a diet supplemented with 200-ppm daidzein (Fonseca and Ward, 2004). Given the affinity of equol to estrogen receptors and the role that estrogen plays in the development of many traits, we hypothesize that daidzein may affect anxiety, social behavior and spatial memory. To address this question we conducted a suite of behavioral tests on male mice given a daidzein-supplemented diet.

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2. Materials and methods

2.1. Animals and standard housing conditions

Adult male Balb/cJ mice (Xian Jiaotong University Laboratory Animal Center, Shaanxi, China) at 6–8 weeks of age upon arrival were housed in groups of five in standard transparent Makrolon cages (42×26×20 cm). Mice were maintained under a reversed light:dark 12:12 cycle (lights on at 20.00 h) and at 21±2 °C; food and water were available ad libitum. All protocols were approved by the Animal Care and Use Committee of Shaanxi Normal University.

2.2. Procedure

Mice were handled daily for three days after a week of habituation. Mice ($n=20$) were randomly assigned to either the daidzein-free diet group (control) or daidzein diet (200 mg daidzein/kg diet; 99% daidzein was obtained from Shaanxi Huike Botanical Development Corporation, Shaanxi, China). Food (Xian Jiaotong University Laboratory Animal Center, Shaanxi, China) was crushed and conglutinated to produce feed for both treatments of the same appearance and hardness. Males were fed these diets for 30 days, after which experimental testing began; they continued to receive the same diet throughout the testing period (Table 1). All behavioral tests were performed daily between 14.00 and 18.00 h and following a sequence designed so that the test of least stress was first. Mouse behavior in the novel cage, open field, elevated plus-maze, and social interaction tests were recorded using a NV-GS15 digital video camera (Panasonic, Tokyo, Japan) and later scored by an observer blind to treatment groups using Observe 5.0 (Noldus, Wageningen, The Netherlands). Activity in the Morris water-maze was recorded and automatically scored by the computer-compatible Morris water-maze tracking system (Shanghai Jiliang Software Technology Co.Ltd, Shanghai, China).

2.3. Body weight and food intake

Body weight was measured before and after consumption of the specified diet for 30 days. Weight gain was determined by subtracting initial body weight from final body weight. A standard amount of food was provided to every cage at the same time daily. Uneaten food was removed and weighed before the provision of new food. Total food consumption for each cage was divided by the number of mice in that cage ($n=5$) to determine individual food intake; this was undertaken daily.

2.4. Open field test

Our open-field test was conducted using a glass box (50×50 cm) comprising a white floor divided into 16 squares and surrounded by a 25 cm high black wall. The arena was illuminated indirectly by two lamps placed above the box but facing outwards and light intensity was approximately 200 lx. Mice were placed individually into the central area of the open field and allowed to explore for 5 min. Time spent in the central and peripheral zones, as well as the number of total transitions, were recorded by NV-GS15 digital video camera (Panasonic, Tokyo, Japan) positioned above the apparatus. The apparatus was cleaned with 70% ethanol after each test.

Table 1
Experimental testing schedule and provision of diet.

Experimental day	Experiment	Diet
1 to 7	Habituation	Regular lab chow
8 to 10	Daily handled	Regular lab chow
11 to 40	Measuring food intakes daily	Specified diet
41 to 47	Novel cage test, open field and elevated plus-maze	Specified diet
48 to 54	Social interaction and male–female social interaction	Specified diet
55 to 61	Morris water-maze	Specified diet

2.5. Elevated plus-maze test

The apparatus consisted of two open arms (30×5 cm) and two enclosed arms (30×5 cm) arranged so that identical arms were opposite each other. The closed arms had 25 cm high black metal walls on both sides but not the short ends, which were open. The arms emerged from a central platform (5×5 cm) and the entire apparatus was raised 50 cm above the floor on four metal legs. The arena was illuminated indirectly by two lamps and light intensity was approximately 50 lx in the open arms. Animals were released into the center of the platform facing one of the open arms and allowed to explore for 5 min. Entry to an arm was defined as the process of entering the arm using all four legs. Time spent in the open and close arms and the number of entries were recorded by NV-GS15 digital video camera (Panasonic, Tokyo, Japan) positioned above the apparatus. After each test the arena was cleaned with 70% ethanol.

2.6. Novel cage test

Males were placed individually into a clean cage identical to their home cage, containing clean sawdust and illuminated with a light intensity of approximately 200 lx. The following behaviors were recorded using a NV-GS15 digital video camera (Panasonic, Tokyo, Japan) for the first 5 min and then analyzed using Observe 5.0 (Noldus, Wageningen, The Netherlands): Wall rearing—the animal stands on its hind limbs and touches the walls of the cage with its forelimbs; rearing—the animal stands on its hind limbs; grooming—fur licking, occasionally using its forepaws and passing them over the nose with a series of brief horizontal movements; digging—digging sawdust with the forelimbs, often kicking it away with the hind limbs; sniffing the environment—sniffing the substrate, cage walls, or air; locomotion—movement around the cage.

2.7. Male–male social interaction

Mice were habituated in the cage used in the novel cage test for 24 h. For our male–male social interaction test, a male stimulus mouse with same weight and derivation then placed on the other side of the cage. Light intensity was approximately 200 lx during the experiment. The total duration and frequency of the following behaviors of the focal male were recorded for the first 5 min using a NV-GS15 digital video camera (Panasonic, Tokyo, Japan) and analyzed using Observe 5.0 (Noldus, Wageningen, The Netherlands): Sniffing the stimulus mouse—head, body, or anogenital region; self-grooming; digging; rearing; wall-rearing; pursuit; locomotion; biting; boxing; escape; sniffing the environment; immobility.

2.8. Male–female social interaction

A female-stimulus mouse of the same age was placed in a cage with the resident male mouse. The total duration and frequency of the following behaviors of the focal male were recorded for the first 5 min using a NV-GS15 digital video camera (Panasonic, Tokyo, Japan) and analyzed using Observe 5.0 (Noldus, Wageningen, The Netherlands): locomotion; immobility; wall-rearing; rearing; sniffing the environment; sniffing the female mouth; sniffing the female body; sniffing the female anogenital region; self-grooming; pursuit; and mounting. Light intensity of the arena floor was 200 lx. After testing, the estrous cycle of the female was determined by slowly lavaging the vagina with 20 µL phosphate-buffered saline and producing a smear. Estrous cycle was

Table 2
Effect of daidzein supplementation on body weight and food intake. Values are mean ± SEM, $n = 10$. *Significant difference between groups ($p<0.05$).

	Control males	Treatment males
Body weight (g)		
Initial	22.26 ± 0.30	22.31 ± 0.37
Final	23.84 ± 0.23	23.52 ± 0.54
Weight gain	1.58 ± 0.24	1.21 ± 0.22
Food intake (g/day)	5.02 ± 0.11*	5.47 ± 0.15*

determined by examining the proportions and morphologies of leukocytes and epithelial cells present in the smear when viewed at 400× magnification using a light microscope (Motic, BA300). Only interactions between males and diestrous females were included in our analysis.

2.9. Morris water-maze test

Spatial learning and memory were assessed using a Morris water-maze task. A circular water tank (120 cm diameter, 45 cm depth) was filled with 22 °C water. A hidden circular platform (10 cm diameter) placed 1 cm below the water surface served as the escape platform. The maximum swimming time permitted for every trial was 90 s, followed by a 20 s rest on the platform. Each mouse was trained for five days (denoted as spatial acquisition training days) with four trials per day. The four starting points were randomized on every day of training. Latency in reaching the platform, swimming speed, distance traveled, and the time spent in the peripheral zone were scored with a digital camera system connected to a computer (Shanghai Jiliang Software Technology, Shanghai, China). On the sixth day, the probe trial was conducted in the absence of the platform and at the same time as trials on the training day. Swimming speed, the number of platform crossings and the time spent in each quadrant were scored with the same digital camera system used above.

2.10. Statistical analysis

All data are presented as mean ± SEM. Results from the open field, novel cage, elevated plus-maze and social interaction tests, and activity during the probe trial of the Morris water-maze test were analyzed using a one-way ANOVA with treatment diet as the between-subjects factor in SPSS version 10 (SPSS Inc., Chicago, USA). The acquisition of spatial memory in the Morris water-maze was analyzed by a repeated-measures two-way ANOVA (specified diets and time as between-subjects factors). The level of significance for all tests was set at 0.05.

3. Results

3.1. Body weight and food intake

We found no significant difference between initial body weight, final body weight and weight gain across the two groups (all $p>0.05$); however, food intake of the daidzein-treated group was higher than the control group ($F_{1,86} = 5.984$, $p = 0.017$) (Table 2).

3.2. Open field

Treatment and control males spent a similar amount of time in the central area ($p>0.05$) but daidzein-treated males were found to cross

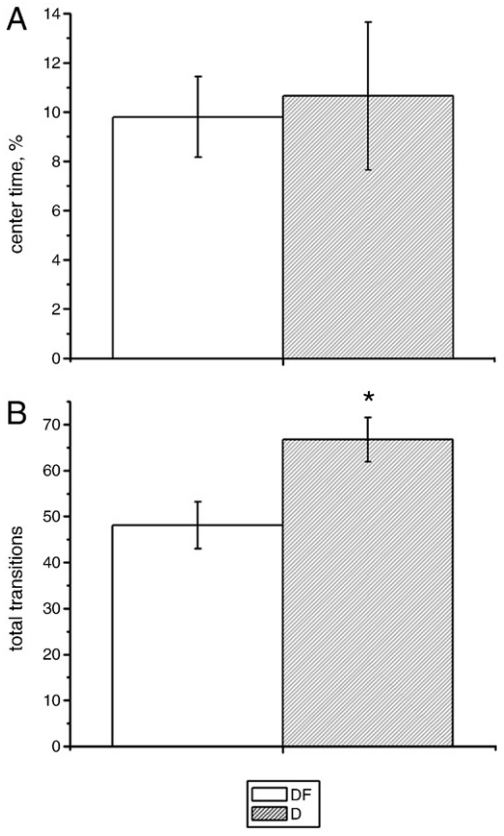

Fig. 1. Percentage of time spent in the central zone (A) and total time of line crossing (B) for males treated (D) and untreated (DF) with daidzein during an open-field test.

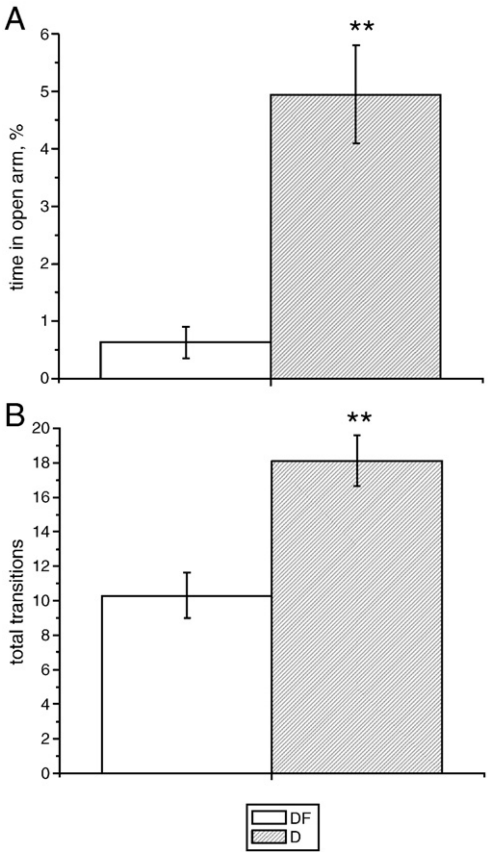

Fig. 2. Proportion of time spent in the open arms (A) and total number of entries in 5 min (B) for males treated (D) and untreated (DF) with daidzein during an elevated plus-maze test. Values are mean ± SEM. *Significant differences between groups ($p<0.05$); **Significant differences between groups ($p<0.01$).

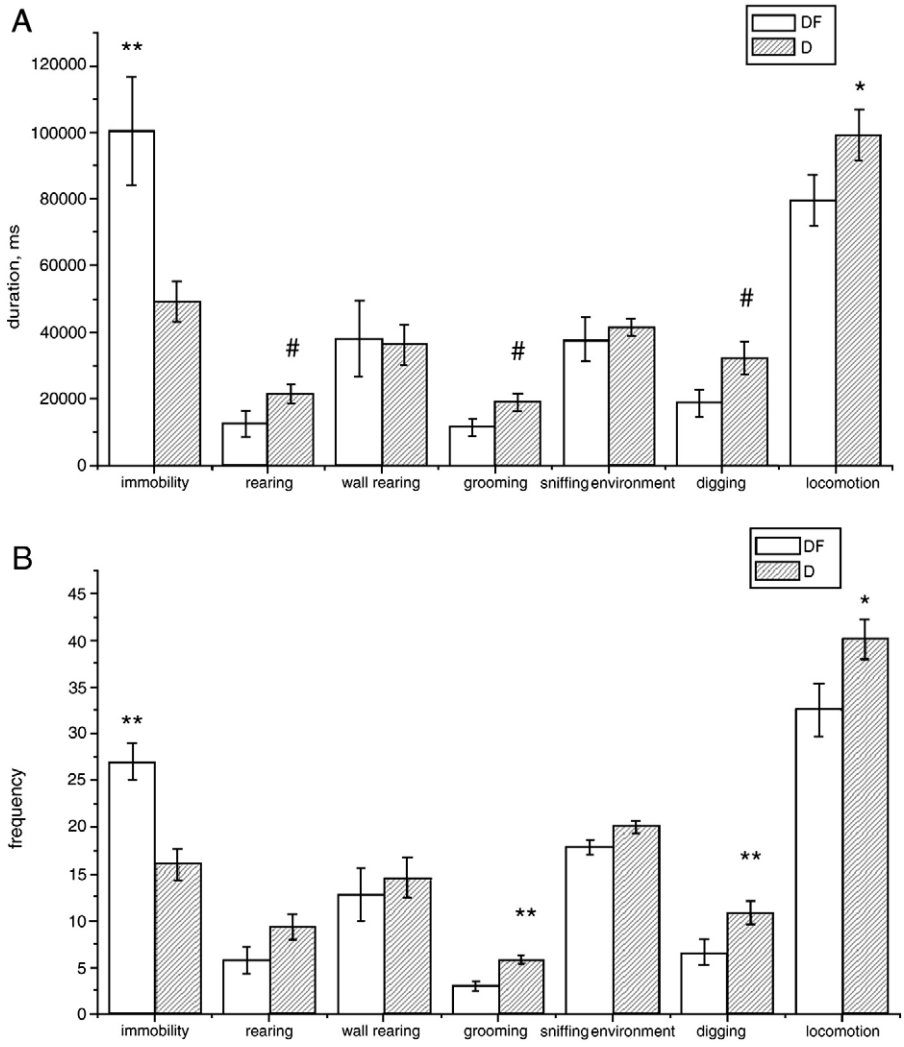

Fig. 3. Duration (A) and frequency (B) of behavior of males treated (D) and untreated (DF) with daidzein during a novel cage test. Values are mean ± SEM, $n = 10$.#Trends between groups ($p<0.1$); *Significant differences between groups ($p<0.05$); **Significant differences between groups ($p<0.01$).

the field more often and showed a greater number of total transitions ($F_{1,14} = 7.007$, $p = 0.019$; Fig. 1).

3.3. Elevated plus-maze test

Daidzein treated males spent a higher proportion of time in the open arms ($F_{1,15} = 16.848$, $p = 0.001$) and made a greater number of transitions ($F_{1,15} = 13.849$, $p = 0.002$) compared to control males (Fig. 2).

3.4. Novel cage test

Daidzein treated males spent more time moving ($F_{1,17} = 5.112$, $p = 0.037$) , and exhibited more bouts of locomotion ($F_{1,17} = 4.606$, $p = 0.047$), self-grooming ($F_{1,17} = 17.734$, $p = 0.001$) and digging ($F_{1,17} = 5.499$, $p = 0.031$) than control males. We also detected an increase trend in the time spent self-grooming ($F_{1,17} = 4.067$, $p = 0.060$, NS), digging ($F_{1,17} = 4.274$, $p = 0.054$, NS) and rearing ($F_{1,17} = 3.414$, $p = 0.082$, NS) by treatment males. No other differences in behavior were found between the two groups (Fig. 3).

3.5. Male–male social interaction

Dietary daidzein was found to increase sniffing behavior (duration: $F_{1,14} = 7.707$, $p = 0.015$; frequency: $F_{1,14} = 17.467$, $p = 0.04$), and to decrease biting (duration: $F_{1,14} = 6.3741$, $p = 0.025$; frequency: $F_{1,14} = 9.308$, $p = 0.009$), and remaining still (duration: $F_{1,14} = 4.477$, $p = 0.046$; frequency: $F_{1,14} = 12.186$, $p = 0.004$). Boxing behavior (duration: $F_{1,14} = 3.784$, $p = 0.072$, NS; frequency: $F_{1,14} = 4.478$, $p = 0.053$, NS) and mouth sniffing (frequency: $F_{1,14} = 3.492$, $p = 0.083$, NS) also tended to decrease in the treatment group compared to the control group (Fig. 4).

3.6. Male–female social interactions

One female-stimulus mouse was found to be in estrus and data from this interaction was excluded from our analysis. We found that treatment with daidzein reduced mounting behavior in the focal male (duration: $F_{1,15} = 8.548$, $p = 0.011$; frequency: $F_{1,15} = 6.652$, $p = 0.021$) and resulted in increased locomotion (frequency: $F_{1,15} = 6.674$, $p = 0.021$) (Fig. 5). We found a trend towards greater wall-rearing in daidzein-treated males, but this did not reach statistical significance (duration: $F_{1,15} = 3.567$, $p = 0.078$, NS; frequency: $F_{1,15} = 4.198$, $p = 0.058$, NS).

3.7. Morris water-maze test

All subjects showed a significant decrease in escape latency during training, indicating successful acquisition of the spatial task. However, we found no effect of our treatment on the acquisition of spatial

memory, swimming speed, swimming distance, or time spent in the peripheral zone (all $p>0.05$; Fig. 6). Memory retention was tested 24 h after the final trial (Fig. 7) in the absence of the escape platform and no significant differences were found for any behavior (all $p>0.05$) except swimming speed ($F_{1,17}=8.066$, $p=0.012$).

4. Discussion

Our findings show that exposure to daidzein in Balb/cJ male mice through food can significantly alter complex behaviors such as locomotor activity, anxiety-related behavior, aggression, and sexual behavior without significant effects on spatial learning and memory. The effects measured here are similar to those of other phytoestrogens and estrogen (Lund and Lephart, 2001; Lund et al., 2001; Lephart et al., 2002; Moore et al., 2004; Wisniewski et al., 2005; Dickerson and Gore, 2007), and we posit that the effect of daidzein on animal behavior may be estrogen-like.

4.1. Locomotor activity

Here, male mice treated with daidzein showed a significant increase in spontaneous locomotor activity when observed in a novel cage (increased locomotion), open field (greater number of total transitions), elevated plus-maze (greater number of total transitions) and Morris water-maze (high swimming speed). Previous research suggests that estrogen receptors, by binding with estrogen-like daidzein or its metabolite, may be involved in the regulation of locomotor activity. Gene disruption of ERα, but not ERβ in male mice significantly increases open field activity (Ogawa et al. 1997, but see Chambers et al., 2007). Gonad-intact female rats exhibit their highest activity levels during proestrus when plasma levels of estrogen are increased (Wollnik and Turek, 1998). Estrogen also greatly potentiated running-wheel activity in both male and female mice, as has also been reported in rats (Ogawa et al., 2003).

Males fed daidzein in our study increased their food intake and this may have lead to obesity as in other studies (Chambers et al., 2007), however, concurrent increases in locomotor activity may have increased energy consumption and acted as an offset. This process may also be associated with binding of daidzein or its metabolites with ERs as it has been shown that site-specific deletion of ERα in a part of the brain involved in body weight regulation highlighted the importance of estrogen activation of ERα in controlling body weight (Musatov et al., 2007). For example, obesity induced by a lack of estrogen activation of ERα may be due to an anabolic process with changes in energy expenditure primarily mediating the weight gain (Musatov et al., 2007). Our finding is consistent with the idea that changes in body mass are primarily due to changes in energy expenditure rather than changes in food intake (Heine et al., 2000).

4.2. Anxiety-related behavior

Daidzein increased the number of visits males made to open arms in the elevated plus-maze and during the male–male social interaction test mice fed with daidzein showed significantly less aggressive behavior and more amicable behavior. We suggest that daidzein produced a significant anxiolytic effect and that this was not due to a change in locomotory behavior as this aspect was unaffected by our treatment. Estrogens and androgens are known to produce marked anxiolytic effects (Bitran et al., 1993; Boissy and Bouissou, 1994; Frye et al., 2000) and exposure to a phytoestrogen-rich diet from gestation up to the point of testing in adulthood also decreases anxiety during elevated plus-maze tests (Lund and Lephart, 2001), as found in the present study.

Mediators of the behavioral effects of gonadal hormones, and possibly phytoestrogens, may be $GABA_A$ receptors and/or the effects of these chemical messengers on GABA activity (Zimmerberg and Farley, 1993; Lucion et al., 1996; Mora and Dussaubat, 1996; Lund and Lephart, 2001). It is noteworthy that the activation of $GABA_A$ receptors or the

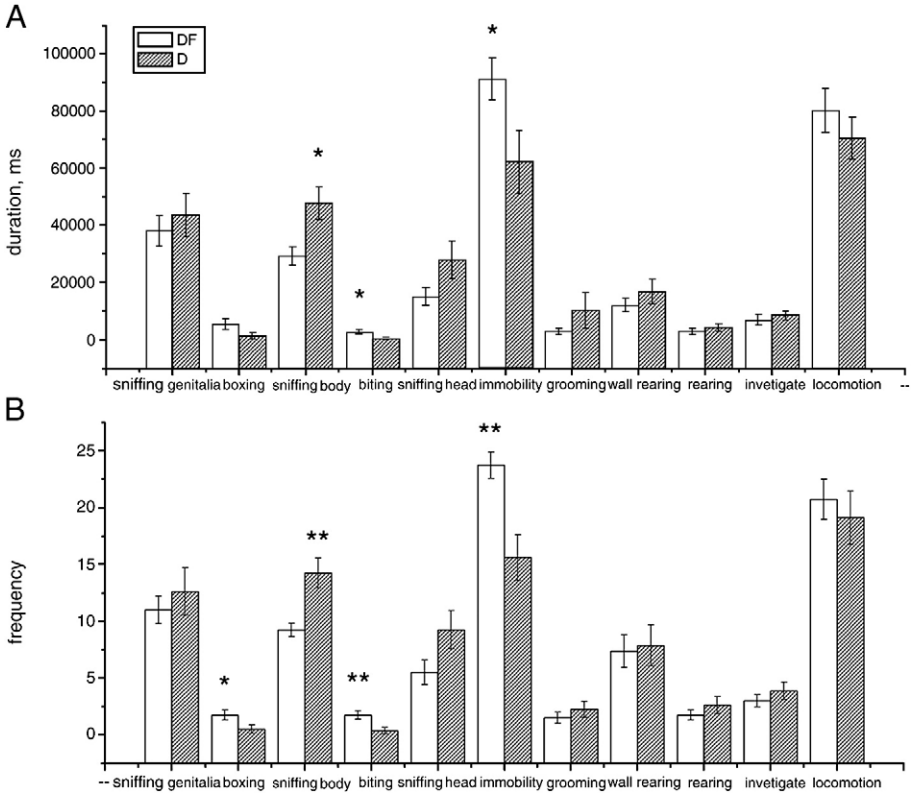

Fig. 4. Duration (A) and frequency (B) of behavior of males treated (D) and untreated (DF) with daidzein during a male–male social interaction test. Values are mean ± SEM. *Significant differences between groups ($p<0.05$); **Significant differences between groups ($p<0.01$).

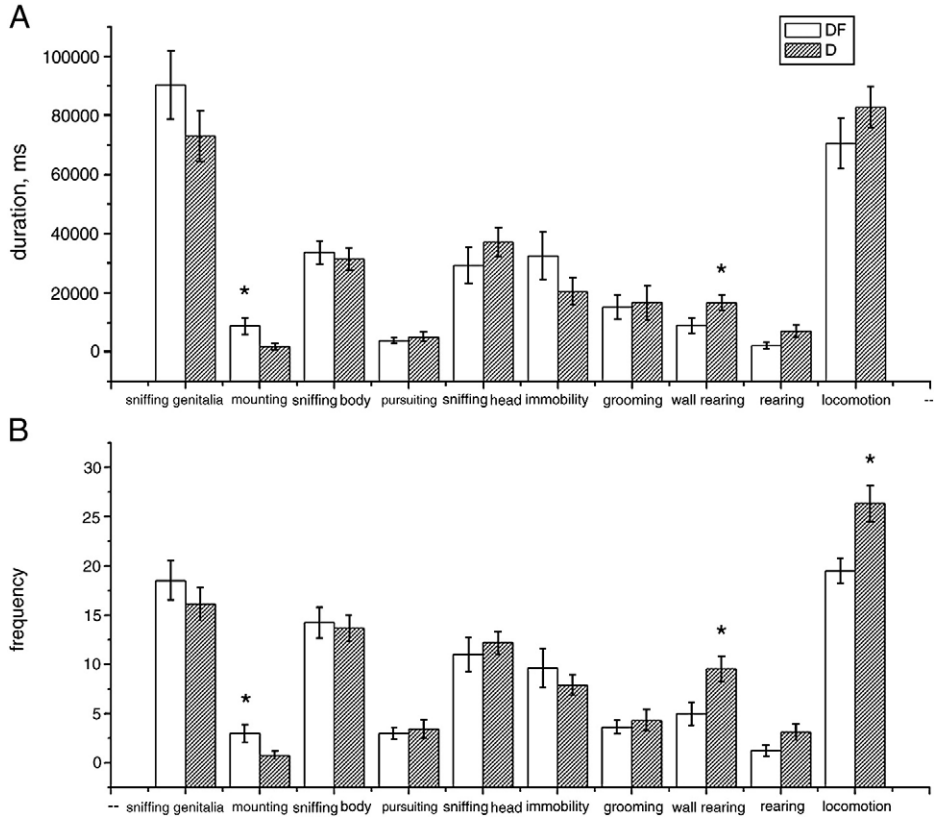

Fig. 5. Duration (A) and frequency (B) of behavior of males treated (D) and untreated (DF) with daidzein during a female–male social interaction test. Values are mean ± SEM. *Significant differences between groups ($p<0.05$).

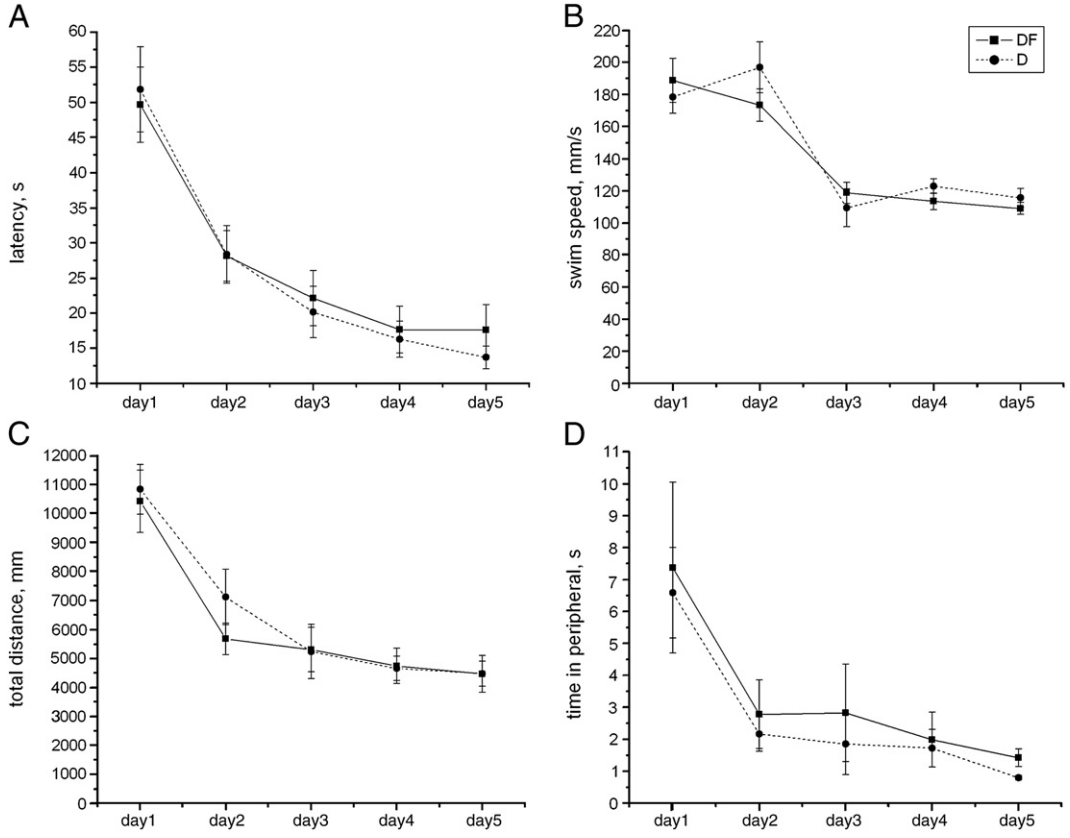

Fig. 6. Activity in the Morris water-maze during spatial acquisition training days. (A) Latency in finding the hidden platform, (B) swim speed, (C) total swimming distance and (D) time spent in the peripheral zone of males treated (D) and untreated (DF) with daidzein. Values are mean ± SEM. *Significant differences between groups ($p<0.05$).

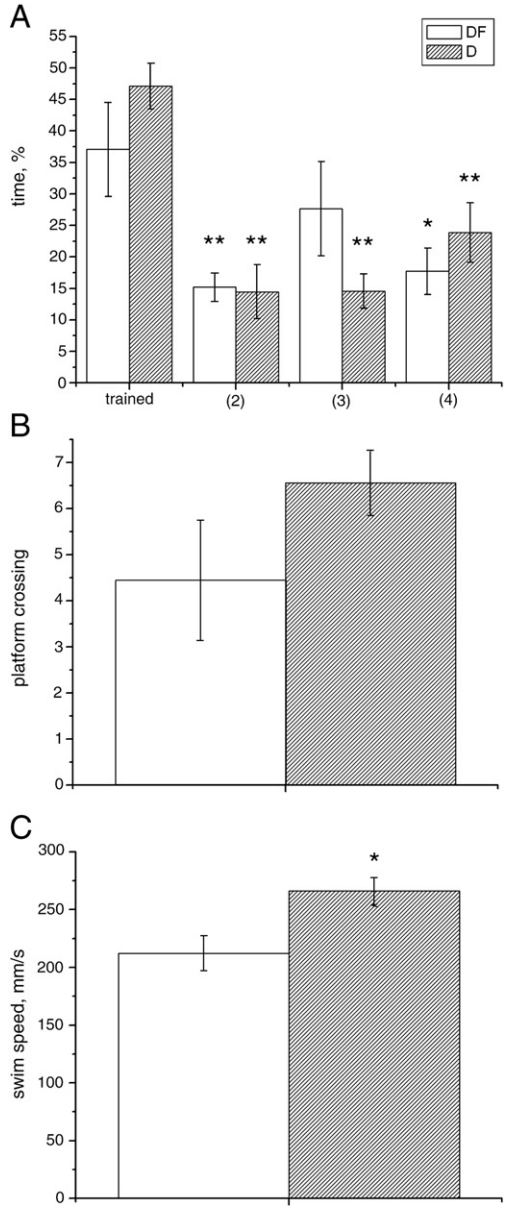

Fig. 7. Activity in the Morris water- maze during the retention test. (A) Proportion of time spent in the four quadrants, (B) platform crossing and (C) swim speed of males treated (D) and untreated (DF) with daidzein. Values are mean ± SEM. *Significant differences between groups ($p<0.05$).

enhanced action of GABA by sex steroids is usually associated with decreased anxiety, coupled with sedation and decreased locomotory activity (Lephart et al., 1996; Lund and Lephart, 2001). However, the actions of estrogen-like molecules such as phytoestrogens and their metabolites, represent complex effects on nonreproductive behaviors such as anxiety, locomotion, spatial learning, and memory (Mora and Dussaubat, 1996; Diaz-Veliz et al., 1997; McCarthy et al., 1997; Lund and Lephart, 2001; Lee et al., 2004, 2005; Patisaul and Bateman, 2008). In this manner, daidzein may produce anxiolytic effects and decrease anxiety via the GABA system. In our study, daidzein did not induce sedative effects but instead increased locomotor and exploratory behavior.

Another mediator of anxiety-like behavior may be ERβ. An agonist of ERβ has been shown to reduce anxiety in adult gonadectomized male and female rats (Walf et al., 2004; Lund et al., 2005). Further, gonadectomized and estrogen-replaced female ERβ-knockout mice spent less time on the open arms of an elevated plus-maze than their wild type counterparts (Imwalle et al., 2005). S-equol has a high and preferential binding affinity for ERβ, whereas R-equol binds much weakly and with a preference for ERα (Muthyala et al., 2004). Therefore, the estrogen-like effects of daidzein may mainly arise from S-equol binding with ERβ. Our findings and those of others suggest that a potential mechanism for the cellular activities of daidzein may be mediated by binding to the nuclear steroid hormone receptor ERβ.

4.3. Aggressive and sexual behavior

Our finding that a diet supplemented with daidzein reduces levels of aggression is novel and add to the growing body of evidence of the effects estrogen and phytoestrogen have on these complex behaviors. When presented with another male, males treated with daidzein engaged in less biting behavior compared to control males. Although in most cases estrogen increases the probability and intensity of aggressive behavior in males, there are exceptions (Simon et al., 2004; Moore et al., 2004; Wisniewski et al., 2005; Clotfelter and Rodriguez, 2006; Patisaul and Bateman, 2008; reviewed in Trainor et al., 2006). Research using phytoestrogen-supplemented diets has also produced mixed results: male monkeys (*Macaca fascicularis*) (Simon et al., 2004) and Syrian hamsters (*M. auratus*) (Moore et al., 2004) have been shown to become more aggressive, while fighting fish (*Betta splendens*) (Clotfelter and Rodriguez, 2006) and mice (Wisniewski et al., 2005) have been observed to be less aggressive. The discrepancy between our experiment and previous research (e.g. monkeys and hamsters) may be due to differences in species, phytoestrogen (daidzein vs other isoflavones) and dose. The mechanism through which daidzein impacts upon aggression may take place via the binding of daidzein or equol to estrogen receptors. For example, male mice lacking ERα (ERα-KO mice) are less aggressive towards their male conspecifics than wild type controls (Ogawa et al., 1997; Ogawa et al., 1998). Further, S-equol binding with ERβ may be responsible for the estrogen-like effects of daidzein and equol also has the further ability to bind with 5 α-dihydrotestosterone, inhibiting this potent androgen and reducing aggressive behavior (Lund et al., 2004). The potential mechanism underlying the regulation of aggression by daidzein requires further investigation.

Our finding of decreased sexual activity (i.e. mounting behavior) following daidzein treatment is consistent with other studies into the effects of estrogens and phytoestrogens (see review by Dickerson and Gore, 2007). For example, coumestrol, an estrogen-like substance fed to rats for the first ten postnatal days affected sexual behavior in males by decreasing mounting and ejaculations (Whitten et al., 1995). Gestational and postnatal exposure to resveratrol throughout lactation also reduced female and male typical sexual behavior (Kubo et al., 2003). These effects are observed in males, but not females, when the exposure is limited to the postnatal lactational period (Henry and Witt, 2006). Here, we show that long-term exposure to daidzein in males also reduces the expression of sexual behavior. Intact and castrated ERα-KO males receiving T replacement have been shown to limit copulatory behavior and in a few cases when ERα-KO males performed, their latencies to display behaviors were longer than wild type littermates (Eddy et al., 1996; Rissman et al., 1997; Wersinger et al., 1997; Wersinger and Rissman, 2000a,b). Decreased sexual behavioral induced by daidzein indicates that daidzein or equol may be an antagonist, and not an agonist, for ERα.

4.4. Spatial learning and memory

It remains unclear whether soy isoflavones positively or negatively affect cognitive functioning in males. Lund and others have reported that the performance of male rats consuming a high-isoflavone diet in a visual–spatial-memory test is poor compared to rats consuming an isoflavone-free diet (Lund et al., 2001). In addition, male rats consuming a lifelong high-isoflavone diet showed poorer performance in

the radial arm-maze test when compared to males switched to an isoflavone-free diet at 80 days of age and fed on the same diet until 120 days of age. However, male rats consuming a soy isoflavone diet have been shown to outperform those consuming an isoflavone-free diet in the spatial delayed matching-to-place test (Lee et al., 2004, 2005). Again, differences in study design, diet and the age of the subjects at testing could explain these inconsistencies or it is possible that soy isoflavones exert either a beneficial or detrimental effect on the cognitive function of males dependent on the duration and/or dosage of treatment.

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