Zoological Studies **50**(1): 24-30 (2011)

Zoological Studies

Differences in Spatial Learning and Memory for Male and Female Mandarin Voles (*Microtus mandarinus*) and BALB/c Mice

Rui Guo, Na Liang, Fa-Dao Tai*, Rui-Yong Wu, Gang Chang, Fen-Qin He, and Qin-Wei Yuan

Institute of Brain and Behavioral Sciences, College of Life Sciences, Shaanxi Normal University, Xi'an, Shaanxi 710062,China

(Accepted September 12, 2010)

Rui Guo, Na Liang, Fa-Dao Tai, Rui-Yong Wu, Gang Chang, Fen-Qin He, and Qin-Wei Yuan (2011) Differences in spatial learning and memory for male and female mandarin voles (*Microtus mandarinus*) and BALB/c mice. *Zoological Studies* **50**(1): 24-30. Spatial learning and memory are important skills for the survival of animals and may vary among species and between sexes according to differences in the use of space. Little is known about spatial learning, memory, and gender differences in mandarin voles (*Microtus mandarinus*), and less is known about how mandarin voles compare to model animals such as BALB/c mice. We assessed spatial learning and memory in male and female mandarin voles and BALB/c mice using a Morris water maze. Analyses revealed significant differences during the learning and memory probe-trials. Mandarin voles performed better than BALB/c mice and showed reduced time spent in the peripheral zone from the 1st to the 5th days. In contrast, BALB/c mice showed increased time spent in the peripheral zone. We also found marked differences between male and female BALB/c mice (males performed better than females), but no such effect in mandarin voles. These data suggest that mandarin voles have good spatial learning and memory abilities and should be considered a model species in future investigations of spatial learning and memory. http://zoolstud.sinica.edu.tw/Journals/50.1/24.pdf

Key words: Mandarin voles, BALB/c mice, Morris water maze.

Within the behavioral neuroscientific fields of neurobiology and biopsychology, the use of animal models enables the study of brain-behavior relations, provides insights into human behavior, and explains underlying neuronal and neuroendocrinological processes (Van der Staay 2006). Animal models, the use of which imitates pathological states occurring in a target species, have significantly contributed to medical progress (De Deyn et al. 2000). Animal models are also research tools for studying pathophysiological mechanisms, in evaluating and developing therapeutic and diagnostic materials and methods, and in education and training (D'Hooge et al. 2001). These species are also useful for identifying the many genetic and environmental factors likely to affect complex behaviors. Increases in nervous system diseases and disorders, and behavioral dysfunctions within modern humans resulting from stress and environmental pollution are important challenges facing research scientists. There is a need for a greater number of appropriate animal models with complex social behaviors in order to study spatial learning and memory.

BALB/c mice are an inbred strain and highly popular in biomedical research, especially in the behavioral neurosciences. BALB/c mice are also widely used in studies of spatial learning and memory performance. For example, Yu et al. (2010) tested spatial learning and the memory ability in adult female BALB/c mice offspring perinatally exposed to daidzein, and Zeng et al. (2010) examined the effect of daidzein on spatial learning in adult male BALB/c mice. Fragopoulou

*To whom correspondence and reprint requests should be addressed. Tel: 86-29-85310286. Fax: 86-29-85310546. E-mail:taifadao@snnu.edu.cn

et al. (2009) used BALB/c mice to investigate the effect of pulsed radiation of 900 MHz from a commercially available mobile phone on spatial learning and memory using a Morris water maze. Harvey et al. (2006) used a novelty-preference paradigm, a task that minimizes performance-related factors such as motivation, in an attempt to examine the effects of lipopolysaccharide on spatial learning and the effect of the anti-inflammatory cytokine, interleukin-10, on learning behaviors in BALB/c mice. The majority of those studies used male BALB/c mice to study spatial learning and memory and rarely considered females. Typically, animals used in behavioral experiments are male, and little is known about whether baseline sexual differences in spatial learning and memory are exhibited by BALB/c mice. Our study therefore aimed to explore possible sexual differences in spatial learning and memory in this strain of mice.

The mandarin vole (*Microtus mandarinus*) is a rodent used in experimental studies that is widely distributed across China. Similar to prairie voles (*M. ochrogaster*), mandarin voles are socially monogamous and exhibit a suite of social behaviors including the formation of long-term pair bonds and biparental care of offspring (Tai and Wang 2001, Tai et al. 2001). The mandarin vole is another interesting model for studying the neurobiological basis of social behaviors. For example, Jia et al. (2008a b) investigated the effects of neonatal oxytocin treatment on aggression, partner preference, and neural activities in mandarin voles. That team also examined the effects of neonatal paternal deprivation or early deprivation on anxiety and social behaviors of adults (Jia et al. 2009). Despite studies on sociality in mandarin voles, characteristics of spatial learning and memory in this species remain unknown, but these abilities are interconnected with social systems. Not surprisingly, whether sexual differences are present in spatial learning and memory in mandarin voles also remains unknown.

Herein, we attempted to assess spatial learning and memory in male and female mandarin voles using a Morris water maze, and then compared vole performances to that of male and female BALB/c mice. We then hypothesized about differences in spatial learning and memory as related to the distinct mating and social systems of these 2 interesting rodent models.

MATERIALS AND METHODS

Subjects

Adult male (n = 17) and female (n = 11) mandarin voles used in this study were laboratory-reared F3-generation animals (30-34 g; approximately 75 d old) originating from wild populations of Henan Province, China. Animals were housed in a temperature-controlled room (21 ± 2°C) and maintained on a light: dark 12: 12-h cycle (lights on at 20:00). During the experiment, they were placed in plastic cages (44 × 22 × 16 cm) with same-sex littermates (3 animals to a cage) with cotton and wood shavings for bedding and nesting material. The light intensity was 200 lux. Water, carrots, and standard rabbit chow (Xi'an Jiaotong Univ. Laboratory Animal Center, Xi'an, China) were provided ad libitum.

Adult male (n = 9) and female (n = 10) BALB/c mice (28-30 g; approximately 75 d old) were purchased from the Xi'an Jiaotong Univ. Laboratory Animal Center. Animals were housed in same-sex groups of 5 animals in conventional plastic cages (42 × 26 × 20 cm). Cotton and wood shavings were provided as bedding and nesting material. Mice were maintained under a constant light: dark 12: 12-h cycle (lights on at 20:00) and at an average temperature of 21 ± 2°C. Water and standard laboratory rat chow (Xi'an Jiaotong Univ. Laboratory Animal Center) were available ad libitum. Prior to testing on the water maze, all mice were habituated to their new environment for 1 wk and were handled for 3 d. All animal handling protocols were in accordance with the *Guidelines for the Care and Use of Laboratory Animals* (Yu et al. 2010) and were approved by the Animal Care and Use Committee of Shaanxi Normal Univ.

Morris water maze

Spatial learning and memory of mandarin voles and BALB/c mice were assessed using a Morris water maze task (Coulbourn Instruments, Whitehall, Pennsylvania, USA). A circular water tank (with a diameter of 100 cm and a depth of 45 cm) was filled with water at 22°C. A hidden circular platform (10 cm in diameter), placed 1 cm below the surface served as the escape platform. The pool was maintained in the same position in the room. Many extra-maze cues surrounding the pool were available to the animals such as equipment, furniture, and several large cardboard figures mounted on the wall; these were in exactly the same position for all trials. The maximum swim time permitted was 90 s, followed by resting for 10 s on the platform. Each animal was trained 4 times a day for 5 d (denoted as the spatial acquisition training days). The interval between 2 training sessions was 1 h. The 4 starting points were randomized on each day of training. Each animal was released from a position opposite the target quadrant and allowed to swim for 90 s. The latency in reaching the platform, swim speed, distance traveled, and time spent in the peripheral zone were scored using a digital camera system connected to a computer (Water Maze 3.20; Coulbourn Instruments, Whitehall, Pennsylvania, USA). On the 6th day, the test (probe trial) was conducted at the same time as training had occurred, in the absence of the platform. Latency and the time taken to cross the platform were scored with the same digital camera and software.

Statistical analysis

Statistical analyses were conducted using SPSS 15.0 (SPSS, Chicago, IL, USA). Data were checked for normality using the one-sample Kolmogorov-Smirnov test. Observations made during the spatial acquisition training period in the Morris water-maze test were analyzed using a repeated-measures two-way analysis of variance (ANOVA; with group and time as between-subject factors) and two-way ANOVA (species × sex). The probe trial of the Morris water maze test was analyzed using a one-way analysis of variance (ANOVA) with the group as a factor, and the number of platform crossings was analyzed using two-way ANOVA (species × sex). Data not normally distributed were compared using nonparametric Kruskal-Wallis tests among the 4 groups and the Mann-Whitney *U* test between 2 groups. All data are presented here as the mean ± SEM. The level of significance was set at $p < 0.05$.

RESULTS

Acquisition

No interaction was found for species and sex for any variable (latency, total distance, swimming speed, or time in the peripheral zone). Mandarin voles showed a decreased escape latency during training, indicating successful acquisition of this spatial task. We found that mandarin voles located the platform more quickly (female: $F_{1,18} = 15.672$, $p = 0.001$; male: $F_{1,25} = 5.005$, $p = 0.034$) and traveled a significantly longer distance (female: $F_{1,18} = 8.645$, $p = 0.009$; male: $F_{1,25} = 7.145$, $p = 0.013$) compared to BALB/c mice (Figs. 1A, B). In addition, the swimming speed was significantly higher in mandarin voles than BALB/c mice (female: $F_{1,18} = 48.929$, $p = 0.000$; male: $F_{1,25} = 29.251$, $p < 0.001$) (Fig. 1C). Again, there were significant species differences only between the 2 female groups ($F_{1,18} = 6.369$, $p = 0.021$) for the time spent in the peripheral zone, as BALB/c mice spent more time (Fig. 1D). There were significant effects of sex in BALB/c mice on the escape latency during training ($F_{1,17} = 6.856$, $p = 0.018$) and time spent in the peripheral zone ($F_{1,17} = 12.225$, $p = 0.003$): females spent more time than males (Figs. 1A, D). In contrast, the swimming speed of male BALB/c mice was faster than that of females ($F_{1,17} = 5.357$, $p = 0.033$) (Fig. 1C). No effect of sex was found for any variable for mandarin voles.

Probe trial

Memory retention was tested 24 h after the final trial in the absence of the escape platform. No interactions between species and sex were found for the number of platform crossings. One-way ANOVA revealed that the number of platform crossings significantly differed among groups ($F_{3,43} = 8.479$, $p < 0.001$) (Fig. 2A). Post-hoc tests showed that mandarin voles crossed the platform more frequently than did BALB/c mice (female: $p < 0.001$; male: $p = 0.028$). Male BALB/c mice made more crossing than female BALB/c mice ($p = 0.022$). Moreover, there was also a significant difference in latency to the platform among groups ($\chi^2_{3,43} = 17.044$, $p = 0.001$) (Fig. 2B). BALB/c mice spent more time seeking the platform compared to mandarin voles (female: $U = 10.000$ $p = 0.002$; male: $U = 30.000$ $p = 0.025$).

DISCUSSION

Sex differences

Male and female mandarin voles performed equally in the Morris water maze. As a socially monogamous rodent, a lack of spatial difference between males and females is expected, as is a minimal difference in brain structure (Trainor 2010). This hypothesis is supported by Gaulin et al. (1989) who suggested that mating system type predicts the presence of a sex difference in maze performance. For example, sex differences in maze learning are generally present in polygamous species but not in monogamous ones. However, 1 study found no effect of sex on the performance in a Morris swim task for either monogamous rodents (*Microtus ochrogaster* and *M. pennsylvanicus*) or a polygamous one (*M. montanus*) (Sawrey 1994).

Here, obvious sex differences were found for the escape latency, time in the peripheral zone, and platform crossings by BALB/c mice, with male BALB/c mice performing better than females. These data are consistent with previous research that male rodents show an advantage in spatial learning in Morris water maze performance (Brandeis 1989) and an 8-arm radial maze (Gresack and Frick 2003, Astur et al. 2004, Jonasson 2005). It seems clear that male BALB/c mice perform better than female BALB/c mice in spatial learning and memory tasks, such as those tested here.

Species comparisons

Our comparison of maze performance across the 2 species showed that mandarin voles performed significantly better at acquisition of spatial tasks and memory retention compared to BALB/c mice. With respect to the escape latency, mandarin voles efficiently acquired the task, and the time to escape gradually decreased during acquisition. For the total distance traveled, a different pattern was found (Fig. 1B): although the swimming distance decreased over time for all subjects, mandarin voles performed better than BALB/c mice overall.

The swimming speed also varied between groups, with mandarin voles swimming faster than BALB/c mice. However, there was a consistent correlation between swimming speed and

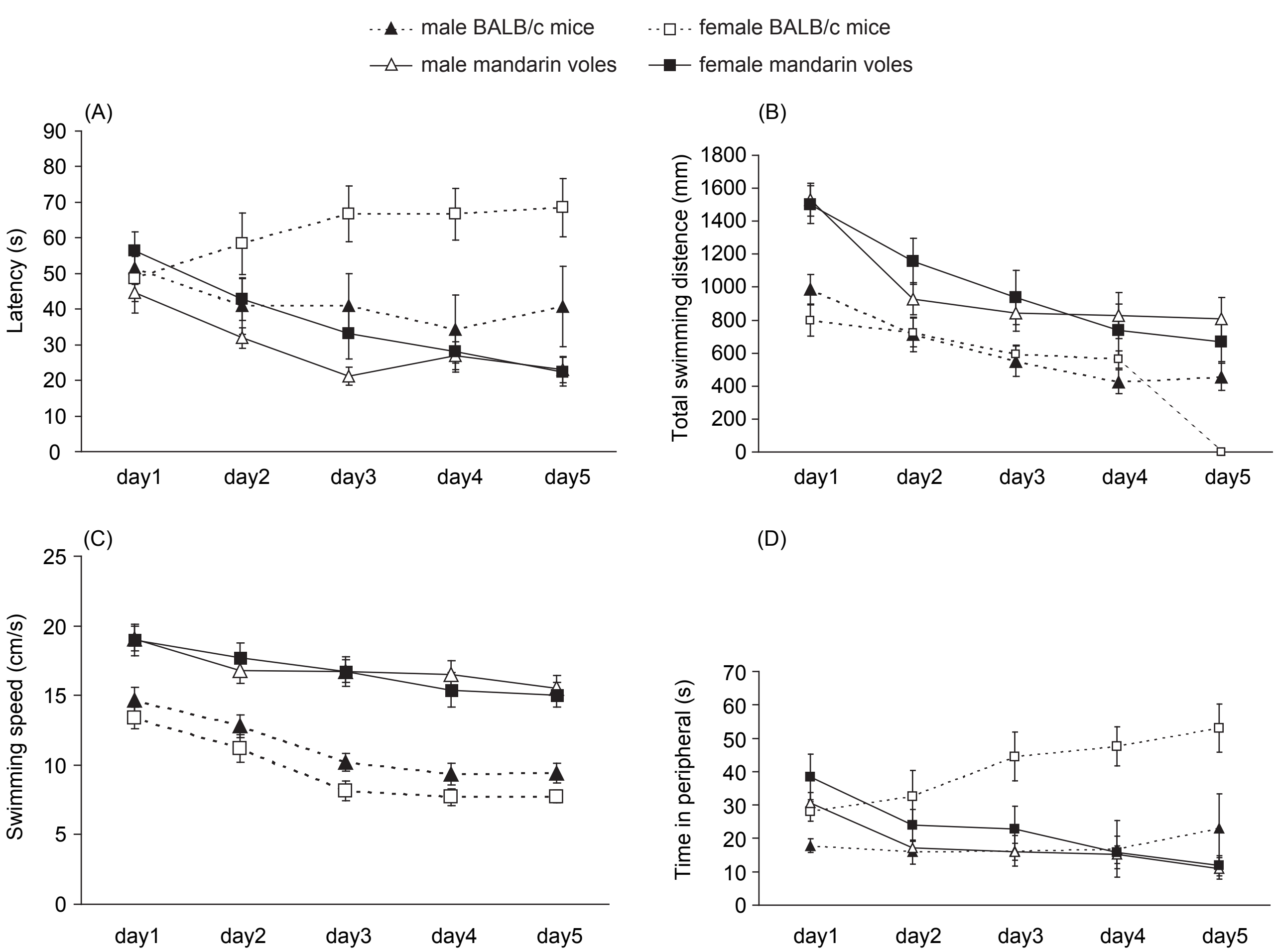

Fig. 1. Activity in the Morris water maze during spatial acquisition training days. (A) Latency to find the hidden platform, (B) total swimming distance, (C) swimming speed, and (D) time spent in the peripheral zone. Values are the mean ± SEM.

distance, and it was suggested that differences in swimming speed may have affected the swimming distance and may subsequently have shortened the latency to the platform. Therefore, studies that draw conclusions based only on the latency to the platform should do so with caution.

Time spent in the peripheral zone differed between rodent species. Previous studies showed that the time in the peripheral zone and learning deficits may be causally related. An increase in the peripheral zone time clearly results in an inefficient acquisition of the water maze task (Cain et al. 1996, Luttgen at al. 2005) and may be due to an increase in anxiety (Treit and Fundytus 1988). The time mandarin voles spent in the peripheral zone decreased from the 1st to the 5th days; in contrast, the time BALB/c mice spent in the peripheral zone increased. Previous research indicated that relative to C57BL/6J and other inbred strains, BALB/c mice show relatively high levels of anxiety-related behavior in an elevated plus maze, open field test, novel object test, and light-dark box test (Crawley and Davis 1982, Bolivar et al. 2000, Bouwknecht and Paylor 2002, Kim et al. 2002, Tang et al. 2002, Francis et al. 2003, Priebe et al. 2005). This may explain the increased peripheral zone time of BALB/c mice. Our analysis of probe trial data, platform crossings, and seeking the platform on the 6th d also led us to posit that mandarin voles are more capable of spatial learning and memory than BALB/c mice.

It is clear that significant species differences exist in the performance variables measured here. Our findings are consistent with earlier reports that BALB/c mice are poor performers in learning tasks and memory retention such with as the Morris water maze (Upchurch 1988, Francis et al. 1996, Klapdor 1996, Crawley et al. 1997, Crawley 2000). Better memory abilities in the mandarin vole may be attributed to its way of life. Mandarin voles live on the Loess Plateau of the north China far from water and effectively have subterranean lives in dark, complicated, underground burrow systems. Vision is probably not very important for mandarin voles because of their small eyes, and spatial learning and memory may play more-important roles in their survival. Therefore, living in a subterranean burrow system may be associated with better memory abilities compared to rodent species living aboveground. It is also possible that vole and mice differences are related to monogamy and nonmonogamy, as these systems affect an animal's capacity for memory consolidation and retrieval. Monogamous and nonmonogamous rodents also differ in rates of brain and behavioral development (Gutierrez et al. 1989), as well as in some neurotransmitter systems such as vasopressin and oxytocin which regulate spatial learning and memory (Engelmann 1996). Some previous reports indicated species differences in spatial learning and memory. For example, Lipp and Wolfer (1998) pointed out that

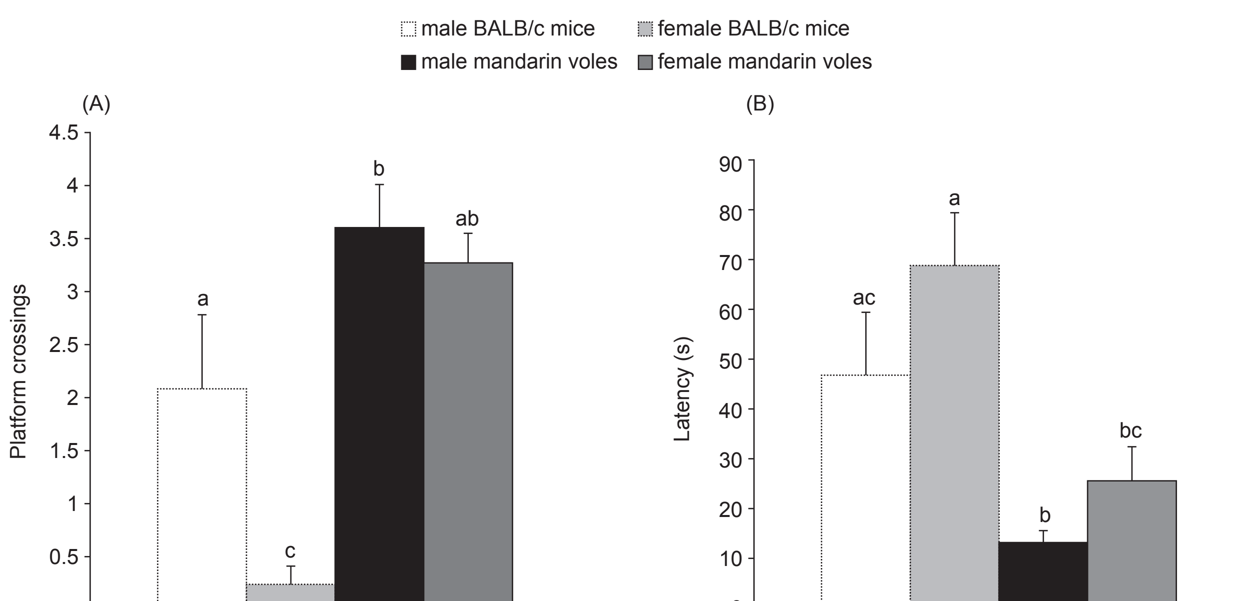

Fig. 2. Activity in the Morris water maze during the retention test. (A) Platform crossing and (B) latency. Values are the mean ± SEM. Groups not sharing the same letter significantly differ.

species differences may exist between rats and mice in the basic cellular and molecular processes of memory. BALB/c mice performed poorly in the Morris water maze because of a variety of visual and neurological defects. In addition to the fact that BALB/c mice are albino, some strain-specific hippocampal and corpus callosum peculiarities were noted (Wahlsten 1989, Lassalle et al. 1994).

During the learning and memory probe-trials, mandarin voles performed better than BALB/c mice. We also found marked sex differences in the performances of male and female BALB/c mice. Mandarin voles did not show significant sexual differences in spatial learning or memory. The species and sexual difference detected here may have been due to different swimming speeds, natural habitats, mating systems, and levels of anxiety. We posited that a high degree of sociability can impose larger adaptive advantages for superior spatial abilities in socially monogamous species. Indeed, other studies highlighted that spatial learning and memory models are reliably and extensively used by BALB/c mice, but similar paradigms are virtually absent from socially monogamous animals. Our results indicate that the direction of sex-specific differences may depend on the type of model and mating system, and that mandarin voles could prove to be an excellent model for studies of spatial learning and memory in socially monogamous systems.

Acknowledgments: We would like to thank G. Chang, R.Y. Wu, F.Q. He, and Q.W. Yuan for their untiring efforts on experimentation and animal husbandry. This research was supported by the National Natural Science Foundation of China (grant nos. 30970370 and 30670273), Fundamental Research Funds for the Central Univ. (GK200901011), and the Natural Science Foundation of Shaanxi Province (SJ08C103).

REFERENCES

Astur RS, J Tropp, S Sava, RT Constable, EJ Markus. 2004. Sex differences and correlations in a virtual Morris water task, a virtual radial arm maze, and mental rotation. Behav. Brain Res. **151:** 103-115.

Bolivar VJ, BJ Caldarone, AA Reilly, L Flaherty. 2000. Habituation of activity in an open field: a survey of inbred strains and F1 hybrids. Behav. Genet. **30:** 285-293.

Bouwknecht JA, R Paylor. 2002. Behavioral and physiological mouse assays for anxiety: a survey in nine mouse strains. Behav. Brain Res. **136:** 489-501.

Brandeis R, Y Brandys, S Yehuda. 1989. The use of the Morris water maze in the study of memory and learning. Int. J. Neurosci. **48:** 29-69.

Cain DP, D Saucier, J Hall, EL Hargreaves, F Boon. 1996. Detailed behavioral analysis of water maze acquisition under APV or CNQX: contribution of sensorimotor disturbances to drug induced acquisition deficits. Behav. Neurosci. **110:** 86-102.

Crawley JN, ed. 2000. What's wrong with my mouse? Behavioral phenotyping of transgenic and knockout mice. 1st ed. New York: Wiley-Liss Press.

Crawley JN, JK Belknap, A Collins, JC Crabbe, W Frankel, N Henderson et al. 1997. Behavioral phenotypes of inbred mouse strains: implications and recommendations for molecular studies. Psychopharmacology **132:** 107-124.

Crawley JN, LG Davis. 1982. Baseline exploratory activity predicts anxiolytic responsiveness to diazepam in five mouse strains. Brain Res. Bull. **8:** 609-612.

De Deyn PP, R D'Hooge, LFM van Zutphen. 2000. Animal models of human disorders - general aspects. Neurosci. Res. Commun. **26:** 141-148.

D'Hooge R, PP De Deyn. 2001. Applications of the Morris water maze in the study of learning and memory. Brain Res. Rev. **36:** 60-90.

Engelmann M, CT Wotjak, I Neumann, M Ludwig, R Landgraf. 1996. Behavioral consequences of intracerebral vasopression and oxytion: focus on learning and memory. Neurosci. Biobehav. Rev. **20:** 341-358.

Fragopoulou AF, P Miltiadous, A Stamatakis, F Stylianopoulou, SL Koussoulakos, LH Margaritis. 2009. Whole body exposure with GSM 900MHz affects spatial memory in mice. Pathophysiology **17:** 179-187.

Francis DD, K Szegda, G Campbell, WD Martin, TR Insel. 2003. Epigenetic sources of behavioral differences in mice. Nat. Neurosci. **6:** 445-446.

Francis DD, MD Zaharia, N Shanks, H Anisman. 1996. Stress induced disturbances in Morris water-maze performance: inter-strain variability. Physiol. Behav. **58:** 57-65.

Gaulin SJC, RW Fitzgerald. 1989. Sexual selection for spatial-learning ability. Anim. Behav. **37:** 322-331.

Gresack JE, KM Frick. 2003. Male mice exhibit better spatial working and reference memory than females in a water-escape radial arm maze task. Brain Res. **982:** 98-107.

Gutierrez P, JS Meyer, M Novak. 1989. Comparison of postnatal brain development in meadow voles (*Microtus pennsylvanicus*) and pine voles (*Microtus pinetorum*). J. Mamm. **70:** 292-299.

Harvey D, R Smith, K English, B Mahon, S Commins. 2006. Interleukin-1 (IL-10) but not lipopolysaccharide (LPS) produces increased motor activity and abnormal exploratory patterns while impairing spatial learning in Balb/c mice. Physiol. Behav. **87:** 842-847.

Jia R, FD Tai, S An, H Broders, XL Ding, Q Kong et al. 2008a. Effects of neonatal oxytocin treatment on aggression and neural activities in mandarin voles. Physiol. Behav. **95:** 56-62.

Jia R, FD Tai, S An, H Broders, R Sun. 2008b. Neonatal manipulation of oxytocin influences the partner preference in mandarin voles (*Microtus mandarinus*). Neuropeptides **42:** 525-533.

Jia R, FD Tai, S An, X Zhang, H Broders. 2009. Effects of neonatal paternal deprivation or early deprivation on anxiety and social behaviors of the adults in mandarin voles. Behav. Process. **82:** 271-278.

Jonasson Z. 2005. Meta-analysis of sex differences in rodent models of learning and memory: a review of behavioral and biological data. Neurosci. Biobehav. Rev. **28:** 811-825.

Kim S, S Lee, S Ryu, J Suk, C Park. 2002. Comparative analysis of the anxiety related behaviors in four inbred mice. Behav. Process. **60:** 181-190.

Klapdor K, FJ van der Staay. 1996. The Morris water-escape task in mice: strain differences and effects of intra-maze contrast and brightness. Physiol. Behav. **60:** 1247-1254.

Lassalle JM, H Halley, P Roullet. 1994. Analysis of behavioral and hippocampal variation in congenic albino and pigmented BALB mice. Behav. Genet. **24:** 161-169.

Lipp H-P, DP Wolfer. 1998. Genetically modified mice and cognition. Curr. Opin. Neurobiol. **8:** 272-280.

Luttgen M, E Elvander, N Madjid, SO Ogren. 2005. Analysis of the role of 5-HT1A receptors in spatial and aversive learning in the rat. Eur. J. Neurosci. **18:** 1652-1659.

Priebe K, WG Brake, RD Romeo, HM Sisti, A Mueller, BS McEwen et al. 2005. Maternal influences on adult stress and anxiety-like behavior in C57BL/6J and BALB/cJ mice: a cross-fostering study. Develop. Psychobiol. **47:** 398-407.

Sawrey DK, JR Keith, RC Backes. 1994. Place learning by three vole species (*Microtus ochrogaster*, *M. montanus*, and *M. pennsylvanicus*) in the Morris swim task. J. Comp Psychol. **108:** 179-188.

Tai FD, TZ Wang. 2001. Social organization of mandarin voles in burrow system. Acta Theriol. Sin. **21:** 50-56.

Tai FD, TZ Wang, YJ Zhao. 2001. Mating system of mandarin vole (*Microtus mandarinus*). Acta Zool. Sin. **47:** 260-267.

Tang X, SM Orchard, LD Sanford. 2002. Home cage activity and behavioral performance in inbred and hybrid mice. Behav. Brain Res. **136:** 555-569.

Trainor BC, EY Takahashi, AL Silva, KK Crean, C Hostetler. 2010. Sex differences in hormonal responses to social conflict in the monogamous California mouse. Horm. Behav. **58:** 506-512.

Treit D, M Fundytus. 1988. Thigmotaxis as a test for anxiolytic activity in rats. Pharmacol. Biochem. Behav. **31:** 959-962.

Upchurch M, JM Wehner. 1988. Differences between inbred strains of mice in Morris water maze performance. Behav. Genet. **18:** 55-68.

Van der Staay FJ. 2006. Animal models of behavioral dysfunctions: basic concepts and classifications, and an evaluation strategy. Brain. Res. Rev. **52:** 131-159.

Wahlsten D. 1989. Deficiency of the corpus callosum: incomplete penetrance and substrain differentiation in BALB/c mice. J. Neurogenet. **5:** 61-76.

Yu CJ, FD Tai, RY Wu, ZZ Song, X Zhang, XL An. 2010. Maternal exposure to daidzein alters behaviour and oestrogen receptor α expression in adult female offspring. Behav. Pharmacol. **21:** 283-291.

Zeng SY, FD Tai, PY Zhai, AF Yuan, R Jia, X Zhang. 2010. Effect of daidzein on anxiety, social behavior and spatial learning in male Balb/cJ mice. Pharmacol. Biochem. Behav. **96:** 16-23.