

Differential Gender-related Vulnerability to Depression Induction and Converging Antidepressant Responses in Rats

Miao-Kun Sun and Daniel L. Alkon

Blanchette Rockefeller Neurosciences Institute, 9601 Medical Center Drive

Academic & Research Building, the 3rd floor, Rockville, MD 20850 USA

The running title: Gender, depression vulnerability & antidepressant treatment

Correspondence and requests for materials should be addressed to:

Miao-Kun Sun, Blanchette Rockefeller Neuroscience Institute, 9601 Medical Center Dr.,
Academic & Research Bldg., Room 319, Rockville, MD 20850, USA; Phone: 301-294-7181;
Fax: 301-294-7007; E-mail: mksun@brni-jhu.org.

The numbers of text pages: 26

tables: 0

figures: 5

references: 42

words in *Abstract:* 222

Introduction: 477

Discussion: 1459

A list of nonstandard abbreviations used:

BDNF, brain-derived neurotrophic factor

HPA, hypothalamic-pituitary-adrenal

OSST: open space swim test

Section: Behavioral Pharmacology

Abstract

Vulnerability of females to depression among humans has not previously been reflected in animal models. Here, we show, by using a novel animal model of depression, that young female Wistar rats are clearly more vulnerable to depression induction than the males. This differential female vulnerability follows estrous cycle stages, is associated with cognitive impairment, and can be markedly transformed with sex hormones. Male hormone reduces vulnerability in females while female hormone increases vulnerability in males. The induced depressive behavior in both sexes, however, is sensitive to imipramine and to the antagonism of the glucocorticoid receptors, within a hormonal pathway previously implicated in human depression. Anisomycin, a protein synthesis inhibitor, eliminates the antidepressive effects of both antidepressants and male hormone, but does not affect depression induction and estradiol-induced higher vulnerability. Moreover, direct cerebroventricular administration of brain-derived neurotrophic factor (BDNF), whose mRNA levels in the hippocampus and frontal cortex reach its lowest levels when estrogen levels are highest, is sufficient to rescue the antidepressive effects in the presence of the protein synthesis inhibitor. A selective enhancement of BDNF expression and/or BDNF signal cascades in the neural circuits controlling mood may represent an effective strategy for the development of novel antidepressants for both sexes, while blocking the gender-related higher vulnerability to potentially depressogenic events may lead to the development of specific antidepressants for the females.

Introduction

Female sex, the personality trait of neuroticism, and depressogenic adversity are three risk factors for major depression in humans (Bebbington, 1996; Kendler et al., 2004). Consistent historically and across cultures, depression, the leading cause of disease-related disability among women in the world, occurs twice as frequently in women as men (Kornstein et al., 2000; Piccinelli and Wilkinson, 2000; Angst et al., 2002; Bebbington et al., 2003; Kessler, 2003). This prominent gender difference in major depression begins in adolescence, prior to which the rates of major depression are equal in girls and boys, suggesting the potential role of estrogen fluctuation in female depression vulnerability (Payne, 2003). The female preponderance of the disorder, however, has heretofore not been reflected in animal models. On the contrary, male animals show more immobility than females in the forced swimming test (Alonso et al., 1991; Contreras et al., 1995; Marvan et al., 1996; Barros and Ferigolo, 1998). The learned helplessness model of depression has also been reported to induce either more escape impairment in male animals (Steenbergen et al., 1989), or no sex-based difference (Setnik et al., 2004).

The novel open space swim test (OSST) used here consists of three daily inescapable trials that induce in rats a non-searching immobility (Sun and Alkon, 2003, 2005). Unlike the forced swim test, the OSST provides sufficient space for rats to swim and develop and execute escape strategies. In addition, no human judgment and scoring are involved. The induced depressive behavior is sensitive to the 3 prototypic classes of antidepressants (prototypic monoamine reuptake inhibitors, monoamine oxidase inhibitors, and atypical antidepressants) and the second-generation antidepressants, the selective serotonin reuptake inhibitors (Sun and Alkon, 2003), but not to buspirone, an anxiolytic (Sun and Alkon, 2004), when the agents are introduced in the depression-induction phase. Chronic treatment of this induced rat immobility with

antidepressants, starting after the immobility behavior is established, also requires weeks of antidepressant treatment to achieve the effectiveness (Sun and Alkon, 2004), a response pattern resembling the human disorder clinically. Thus, the OSST provides an animal model in which long-term effects of antidepressants can be followed and determined individually (Sun and Alkon, 2004), including a direct evaluation of whether a particular antidepressant treatment can actually cure the disorder maintenance of behavioral recovery after discontinuation of the treatment). In this study, we further investigate whether rats exhibit a gender and estrous cycle-dependent differential vulnerability, and if so, whether sex and stress-related hormones and neurotrophic factor may be involved. Our results show that there is a gender- and estrous cycle stage-dependent difference in vulnerability to OSST induction of the depressive behavior in rats and that the induced depressive behavior is associated with impaired performance in the passive avoidance task. The induced depressive behavior in the males and females, however, converges on its sensitivity to glucocorticoid receptor antagonism, inhibition of protein synthesis, and antidepressant treatment.

Materials and Methods

Male and female Wistar rats (Charles River Laboratories) of about 2 months old (200-250 gm) were used in the study. They were housed in a temperature-controlled (20-24°C) room for at least a week prior to experimentation, allowed free access to food and water, and kept on a 12-hour light/dark cycle. They were randomly assigned to different groups (8 rats/group) and moved to the test room in their home cages at least 1 hour before the OSST trials or learning and memory task sessions.

Open Space Swim Test (OSST)

Rats were placed individually in a round pool, which had a diameter of 152 cm and height of 60 cm and was filled with 40 cm H₂O (24 ± 1 °C). No escape was provided in the OSST trials. Rats were free to swim (or not to) for 7, 11, or 15 minutes (the particular set of trial length for each group) and were then returned to their home cages after drying. The observer(s) were obscured from sight of the rats, but were able to observe the animals' behaviors on-line (Video Monitor BWM9, Javelin Electronics Inc.). The same procedure with the same set of trial length for each group was followed 24 hours later for 2 more days. For instance, the OSST trials for 11-min groups consisted of 3 consecutive trials at a frequency of 1 trial per day (11-min/trial). The swimming/drifted path and distance were recorded with a video-tracking system (Poly-Track Video Tracking System, San Diego Instruments, Inc.). Imipramine was administered (10 mg/kg, i.p.) 23, 3, and 1 hr before the 2nd and 3rd OSST trials. To determine a potential involvement of sex hormones in the gender-related vulnerability to OSST, we evaluated the effects of male sex hormone in females and female sex hormone in males on their sensitivity to OSST, respectively. Estradiol or androsterone was administered at 200 µg (s.c.), starting 8 days before the first OSST

trial. This was followed with 2 more doses administered every third day after the previous injection, with the last (the third) dose administered the day before the first OSST trial. Mifepristone and spironolactone (20 mg/kg, i.p.) were administered immediately after the first trial and 1 h before the 2nd and 3rd trials. Anisomycin was injected (40 mg/kg, i.p.) immediately after the 1st and 2nd trials. BDNF was administered bilaterally (1 µg/µl/side, i.c.v.) about 0.5 h after the 1st and 2nd trials. For i.c.v. injections, agents were solubilized in artificial cerebrospinal fluid consisting of 125 mM NaCl, 3 mM KCL, 1.3 mM MgSO₄, 2.4 mM CaCl₂, 26 mM NaHCO₃, 1.25 NaH₂PO₄, and 10 mM glucose. Estradiol, mifepristone, and spironolactone were solubilized in DMSO and diluted/suspended with 2-fold volume of saline. The other pharmacological agents were solubilized in saline. The control rats received appropriate vehicle injections.

A short OSST version consisting of three 400 s trials at intervals of 2 h between the trials was used for an evaluation of the vulnerability of female rats to depression induction at a particular estrous cycle stage. In this test, imipramine was administered at 15 and 10 mg/kg (i.p.) 1 hr before the 2nd and 3rd trials, respectively.

Estrous Cycle Stage Verification

In the short OSST version test, which was completed in a day, female rats were used after at least one, and usually two complete and regular 4-day estrous cycles, determined daily and followed for each rat. Vaginal samples were collected between 12:00 and 13:00, by gently immersing a sterile cotton-tipped applicator in physiological saline. Animals with vaginal smears that contained predominantly leukocytes ($\geq 60\%$) and numerous large nucleated epithelial cells were classified as metoestrous. Diestrous smears contained relatively few cells of any type (\geq

60% leukocytes and occasional nucleated epithelial cells). Smears that contained primarily nucleated epithelial cells ($\geq 60\%$) and few leukocytes ($\leq 10\%$) were classified as proestrous. Smears that contained primarily cornified cells ($\geq 90\%$) were classified as estrous.

Passive Avoidance Learning and Memory Task

An avoidance apparatus (Gemini II Avoidance System, San Diego Instruments, Inc, San Diego) was used for the multi-trial passive avoidance task (Sun and Alkon, 2004). The task consisted of two trials: an acquisition trial and a retention trial. In the acquisition trial, 24 h after the last OSST trial, the rat was placed individually in the compartment on the right side (the initial compartment). The inter-compartment door was opened after a 60 s acclimation period. At the same time the house light and tone in the initial compartment were also automatically switched on. If the rat stepped into the dark compartment on the left side (the dark compartment), the door was closed automatically and an inescapable foot shock (0.3 mA/2 s) was delivered through the grid floor of the dark compartment. After receiving the foot shock, the rat was returned to the initial compartment. The same acquisition procedure was repeated until the rat stayed in the initial compartment for 120 s. The number of training trials needed for the rat to stay in the initial compartment for 120 s was used as the index of learning acquisition.

The retention trial started 24 hours after the end of the acquisition trial. Each rat was placed individually in the initial compartment. The door was opened after a 60 s acclimation period, with house light and tone being switched on automatically at the same time. The step-through latency in the retention trial (with a maximum 300 s cut-off time) was used as the index of retention of the learned experience.

For evaluation of the effects of antidepressants and other agents on depression-related impact on the multi-trial passive avoidance task, all agents were administered during the OSST phase (imipramine) or pre-OSST trials (estradiol and androsterone), as described above. Agents-only control groups received the same doses of agents with the same injection schedules as the test groups. These control rats were also brought to the OSST trial room (though without subjecting to the OSST trials) during the OSST trial days. No agents were administered during the multi-trial passive avoidance task.

***In vivo* Cannulation**

Rats were anesthetized with sodium pentobarbital (60 mg/kg, i.p. with supplemental doses if necessary) and placed in a stereotactic apparatus (Kopf Instruments, Tujunga, CA). The core temperature of rats was monitored and kept constant (38.0 ± 0.5 °C) with a warming light. Two stainless steel guide cannulas were placed with the tips positioned at the coordinates (anterior-posterior, 0.5 mm; lateral 1.5 mm; horizontal, 3.2 mm), under aseptic conditions. At the end of surgery and under appropriate anesthesia, rats received (s.c.) banamine (1 mg/kg) and ketoprofen (5mg/kg). A 7-day recovery period from surgery was allowed. For i.c.v. injection, the injection cannula was connected via PE20 tubing to a Hamilton microsyringe. The injection was slow and in small volume (1 μ l/2 min), using a microinjection pump.

All procedures were conducted according to National Institutes of Health Animal Care and Use Committee guidelines and approved by the Ethical Committee of the Institute. Statistical analysis was performed using the analysis of variance (ANOVA), followed by Newman-Keuls multiple comparisons test. $P < 0.05$ was considered significant.

Results

Gender-dependent vulnerability to depression induction, related learning and memory impairments, and effects of antidepressant treatment

The impact of different lengths of OSST trials (thus intensity of the potentially depressogenic events) on the mobility was determined in male and female rats. In the OSST, female rats showed clearly higher vulnerability to depression induction than the males. The eleven-min/trial (one 11-min/trial/day for 3 days) was not sufficient in the males to induce the non-searching immobility behavior ($F_{2,23} = 0.275$, $p > 0.05$ over trials; Fig. 1a), but induced a significant reduction in mobility in the females ($F_{2,23} = 40.718$, $p < 0.001$; Fig. 1b). There were significant effects of groups, trials, groups x sex, groups x trials, and groups x sex x trials (all with $p < 0.001$). The induced immobility in both sexes was sensitive to the administration of imipramine, a clinically effective, prototypical tricyclic antidepressant. Imipramine treatment significantly reduced the immobility that was induced with 15-min/trial in the males, with 11-min/trial or 15-min/trial sessions in the females, while the drug did not significantly affect the mobility in the male 11-min/trial group, in which depressive behavior was not induced. The seven-min/trial (one 7-min/trial/day for 3 days) did not induce the immobility in the male or female rats (all with $p > 0.05$ over trials; Fig. 1). The net distance moved for the same trial durations did not differ statistically between groups for the first trial (all with $P > 0.05$) and thus not presented in details. The distance value for the first trial was calculated as 100% for a particular animal. The swim distance has previously been found to reliably reflect duration of mobility during the test (Sun and Alkon, 2003).

This induced depressive behavior also had substantially negative impact on cognitive capacity as evaluated in the multi-trial passive avoidance task. This learning deficit, however,

occurred only after the males were exposed to 15-min/trial ($F_{1,15} = 20.362$, $p < 0.001$; Fig. 2a), but not after the 11-min/trial OSST, while such impairment was observed in the females after the 11-min/trial ($F_{1,15} = 15.568$, $p < 0.01$; Fig. 2b) and 15-min/trial ($F_{1,15} = 24.262$, $p < 0.001$; Fig. 2b). There were significant effects of groups ($F_{7,112} = 16.971$, $p < 0.001$) and groups x sex ($F_{7,112} = 8.522$, $p < 0.001$). No learning and memory deficits were observed in the male and female groups after the 7-min/trial OSST (not shown).

The impairment in the learning was not only related to the induced depressive behavior, but was also sensitive to imipramine treatment during the OSST phase. Imipramine itself did not significantly affect the learning in either sex when the agent was administered at the same doses and with the same injection schedules as the test groups. These agent-control rats were not subject to the OSST trials. Nor did imipramine itself have significant effect on the learning in the males after the 11-min OSST trials, in which immobility was not induced. However, it eliminated the learning impairment in the male rats that had 15-min OSST trials (Fig. 2a) and in the female rats that had 11-min or 15-min OSST trials (Fig. 2b). The learning performance of these rats with imipramine treatment during the OSST trial phase did not differ from their corresponding control groups (all with $p > 0.05$).

The retention test of the multi-trial passive avoidance task further revealed that depressed rats exhibited memory deficits (Male 15-min group: $F_{1,15} = 5.300$, female 11-min group: $F_{1,15} = 5.216$, female 15-min group: $F_{1,15} = 5.167$; all with $p < 0.05$, vs. their controls; Fig. 2c,d), although these rats received more training trials during the acquisition. The depression-related memory deficits were sensitive to imipramine treatment during the OSST trial phase, revealing no significant difference in the memory performance of the male 15-min trial and female 11-min and 15-min trial groups with the imipramine treatment during the OSST trial phase, as compared

with their corresponding control groups (all with $p > 0.05$; Fig. 2c,d). Imipramine treatment itself (without the OSST trials) did not affect the recall in the task for either sex. Nor did it have a significant effect on the recall in the males after the 11-min OSST trials, in which immobility was not induced.

Sensitivity of gender-dependent depression vulnerability to sex hormones and imipramine treatment

To evaluate the involvement of underlying signal cascade in the female vulnerability to depression induction, we used several additional approaches. Given this clear female vulnerability to the OSST depression model, we directly evaluated roles of sex hormones in the differential vulnerabilities to the induction of the depressive behavior. After an eight-day treatment with estradiol, an estrogen receptor agonist, male rats became more vulnerable to a subsequent OSST (Male 11-min-estradiol group: $F_{1,15} = 5.689$, $p < 0.05$, vs. male 11-min group; Fig 1a) and depression-related cognitive deficits (Fig. 2a,c). This increased vulnerability was sensitive to imipramine treatment since no significant decrease in mobility over trials was observed with imipramine treatment (male 11 minutes/trial, estradiol+imipramine; $F_{2,21} = 0.414$, $p > 0.05$; not shown). Administration of androsterone, an androgen receptor agonist, to female rats for the same duration, on the other hand, eliminated their higher vulnerability to depression induction (Female 11-min-androsterone group: $F_{1,15} = 4.582$, $p < 0.05$, vs. female 11-min group; Fig. 1b) and the depression-related cognitive deficits (Fig. 2b,d), while the hormones themselves at the same dose and with the same administration schedule in rats that were not subject to the OSST trials did not have significant impact on the learning and memory (Fig. 2).

Depression vulnerability across the estrous cycle

We directly evaluated whether the vulnerability in females to the induction of the immobility behavior fluctuated across the estrous cycle stages. A 3-day trial session in rats lasted almost the entire estrous cycle in the animal. A short test was therefore developed to evaluate differences in depression vulnerability at various estrous cycle stages. Three 400 s trials at 2 h intervals revealed that proestrous and estrous females had higher vulnerability to the depression induction, as compared to metoestrous and diestrous females (groups: $F_{3,84} = 6.109$, $p < 0.001$; Fig. 3), and as compared to the males ($F_{4,105} = 4.652$, $p < 0.001$; Fig. 3). The female rats at the 4 estrous cycle stages, however, all showed significant decrease in mobility over trials (all with $p < 0.05$), with responses eliminated after 2 imipramine doses applied 1 h before the 2nd (15 mg/kg, i.p.) and 3rd (10 mg/kg, i.p.) OSST trials (not shown). The male rats, on the other hand, did not show significant change in mobility over the trials in response to the same intensity of the trial events ($F_{2,21} = 0.009$, $p > 0.05$; Fig. 3).

Converging effects of a glucocorticoid receptor antagonist on the depression induction

We directly evaluated whether the OSST-induced decrease in mobility was sensitive to blocking the glucocorticoid receptors in the three 1 trial/day OSST. Administration of mifepristone, a glucocorticoid receptor antagonist, eliminated the decrease in mobility in either sex (male-15 min groups: $F_{2,63} = 3.231$, $p < 0.05$; Fig. 4a; female-11 min groups: $F_{2,63} = 3.166$, $p < 0.05$; Fig. 4b), while spironolactone, a mineralocorticoid receptor antagonist, was ineffective in both sexes ($p > 0.05$; Fig. 4).

Converging effects of an inhibitor of novel protein synthesis and brain-derived neurotrophic factor

The impact of inhibiting protein synthesis (protein synthesis-dependent synaptic/structural remodeling) on immobility induction was evaluated. Interestingly, the induction of immobility in both sexes and estradiol-induced higher vulnerability in the males were not changed by anisomycin, a specific inhibitor of protein synthesis (Fig. 5), while antidepressive effects (imipramine in both sexes and androsterone in females) were dependent on novel protein synthesis, since their effects on the mobility did not occur with the co-administration of anisomycin. The magnitudes of immobility over trials thus did not differ in the male ($F_{3,84} = 0.625, p > 0.05$) and female groups ($F_{3,84} = 0.387, p > 0.05$).

While the co-administration of anisomycin effectively blocked the antidepressant effects of imipramine in both sexes and androsterone in the females, intracerebroventricular administration of BDNF completely reversed the blockade produced by anisomycin in both sexes (anisomycin+BDNF vs. anisomycin in males: $F_{1,15} = 18.726, p < 0.001$; and in females: $F_{1,15} = 17.118, p < 0.001$; Fig. 5). No significant changes in mobility over trials were observed in BDNF groups of either sex ($p > 0.05$; Fig. 5). These results are in line with the evidence reported by others that estrous cycle stages- and stress-related hormones decrease the expression of BDNF in the hippocampus and the prefrontal cortex, that antidepressant treatment increases the production of BDNF (Russo-Neustadt et al., 2000), and that central administration of BDNF produces antidepressant-like behavioral effects (Shirayama et al., 2002).

Discussion

Our findings indicate that female rats exhibit a higher vulnerability to OSST than the males by increasing susceptibility to depressogenic effects of adversity. The three 11-min/trial OSST was not sufficient to induce an increased immobility over trials in the males but was strong enough to trigger the increased depressive behavior over trials in the females. This observation is consistent with the report that the female preponderance in depression cannot be explained by sociodemographic factors (Klose and Jacobi, 2004). Furthermore, our results show that the higher female vulnerability fluctuates across estrous cycle stages and is, at least partially, mediated by the sex hormones. Agents that specifically target the molecular/synaptic signal pathways underlying the gender-specific mood deregulation may have therapeutic values as effective antidepressants for the treatment of depression associated with large estrogen fluctuations. On the other hand, the induced depressive behavior and effects of antidepressants in both sexes appear to share some common regulatory pathways and pathology. First, the induced depressive behavior in either sex is sensitive to imipramine, a prototypical tricyclic antidepressant, and a blockade of the glucocorticoid receptors. The higher vulnerability to depressogenic events in the females thus does not possess antidepressant-resistant profiles and may also involve the same HPA responses. Second, the adverse impact on learning and memory depends on the induced behavioral changes, in that impaired learning and memory occur only when immobility is induced, though the behavioral change was induced at different intensity of the depressogenic events in the females and the males. Third, the induced depressive behavior in either sex shows an apparently identical underlying phenomenon: its induction is independent of novel protein synthesis, while the antidepressant effects depend on novel protein synthesis. Nevertheless, these observations are consistent with the view that a unified basis for the

development of the mood disorders must exist (Hattori et al., 2005), since they occur in both men and women.

It is rather interesting that the OSST induced a gender-related differential vulnerability to the induction of the depressive behavior, while such difference has not been observed with the forced swim test. One possible explanation for the difference between the OSST and the forced swim test may stem from searching activity in which rats can engage. The OSST setup is the same as that used for water maze spatial learning and memory (but without the escape), an environment in which rats are known for their expertise in developing spatial and procedural escape strategies. In the large pool, rats plan and execute (probably many) escape strategies, judging from their searching activity and active swimming. However, their strategies are bound to fail since no escape is provided. Defeat or despair-like responses (immobility), with hopeless expectancies, gradually predominate, consistent with the observation that a certain length of trials was required for the rats to develop the immobility. Three 7-min trials over 3 days (1 trial/day), for instance, were below the threshold in either sex to develop the behavior. The mood change is thus consistent with the learned hopelessness type of response in humans, a characteristic symptom of depression (Abela, 2001). Although the same type of behavior (immobility) was induced in the OSST, the underlying mechanisms and molecular cascades may differ from those induced with the forced swim test. For instance, one noted difference is that the depressive behavior induced with OSST is more sensitive to antidepressants (Sun and Alkon, 2003).

It had not been clearly established previously whether female sex hormones may play any role in depression vulnerability and what might be the underlying mechanism if such a role does exist. The gender and estrous differences could result from higher estradiol levels and associated

stress-related hypothalamic-pituitary-adrenal (HPA) axis activity in females, most dramatic at proestrous and estrous stages (Miller et al., 2004; Rhodes et al., 2004). The difference in HPA activity has been tracked to circulating estrogen (Burgess and Handa, 1992). Fluctuating activities of estrogen and HPA activities are accompanied by changed activity of neurotrophins, particularly BDNF, which may play a central role in the efficacy of antidepressants (Nestler et al., 2002; Castrén, 2004), synaptic remodeling, and associated neuronal survival/neurogenesis (Santarelli et al., 2003; Sairanen et al., 2005). BDNF mRNA levels in rat hippocampus and prefrontal cortex fluctuate significantly across the estrous cycle stages, reaching their lowest levels during the proestrous stage (Gibbs, 1998) or the estrous stage (Cavus and Duman, 2003) when estrogen levels are the highest. Consistent with these findings, glucocorticoids (e.g., cortisol, corticosterone) suppress BDNF expression. Furthermore, in males, testosterone inhibits the HPA axis by activating central androgen receptors (Handa et al., 1994; Viau, 2002), and BDNF functions as the neuronal survival mediator of testosterone (Rasika et al., 1999).

The induced depressive behavior is accompanied by impaired learning and memory in the multi-trial passive avoidance task in both sexes, responses that are sensitive to the imipramine treatment during the OSST trial phase. Its potential long-term impact on the learning and sensitivity to chronic antidepressant treatment remain to be studied. The observation differs from the forced swim test in that the most immobile induced with the forced swim test appear to be the “smartest” (West, 1990). The multi-trial passive avoidance task involves an entirely different context (pool vs. compartments) and environment (in a different room) from those of the OSST so that the results are unlikely to be affected by contextual/environmental similarity (such as if examined with water maze spatial learning and memory task). Similarly, the effects of the OSST trials and antidepressant treatment on learning and memory cannot be attributed to non-specific

changes in sensory and motor ability of the rats. First, if the OSST trials impaired the sensory and motor ability of the rats, the results would have been the opposite. A direct evaluation of the sensory and motor ability of the rats with a visible platform test after the OSST trials reveals no obvious difference between the OSST rats with or without imipramine treatment and control rats (Sun and Alkon, 2004). The imipramine was administered at the well-established doses at which it does not induce changes in non-specific locomotor activity in rats (Bai et al., 2001; Krocza et al., 2001; Takamori et al., 2001; Kitamura et al., 2002). The learning and memory changes observed in the study are thus unlikely to be caused by a non-specific effect of the OSST and/or drug treatment on sensory and motor ability of the animals. The impact of the male sex hormone at the administered doses on the learning ability of female is relatively big (more than 30% on average after the 11-min OSST). The group difference in passive avoidance learning induced with estradiol and 11-min OSST in the males, however, appears relatively small (less than 20% on average). It is not clear whether this is due to insufficient doses of estradiol administered in our study to overcome the impact of male hormone present in the males.

Surprisingly, the induction of depressive behavior is not affected by anisomycin. Anisomycin is a potent inhibitor of mRNA translation via interference with transpeptidation and has been used successfully at the identical dose in a number of behavioral paradigms to determine whether novel protein synthesis is involved (Lattal and Abel, 2001; Pang et al., 2004; Inda et al., 2005). Our results suggest that the development of the depressive behavior does not involve novel protein-dependent synaptic/structural remodeling, although stress is known to cause neuroadaptations in the brain (McEwen and Sapolsky, 1995). Maintenance of a healthy mood and expression of antidepressant action, on the contrary, appear to require novel protein synthesis. Antidepressants depend on novel protein synthesis and may depend on BDNF

mediated synaptic/structural remodeling and/or an active maintenance of such components. These results suggest a potential key role for BDNF, whose levels fluctuate across the estrous cycle and differ in gender-related HPA responses, in the gender and estrous cycle-correlated higher vulnerability to induction of immobility behavior. Our results suggest that BDNF is effective in all stages of the estrous cycle of the females since its antidepressant effects were observed in the rats with mixed stages of the estrous cycle and with trial sessions across almost the entire estrous cycle. At its doses administered in the present study, BDNF is equally effective in the males and females. It remains, however, to be determined whether a baseline difference between the males and the females can be revealed, probably by using smaller BDNF doses. Because of its close parallels with human female vulnerability to depression, this OSST model of depressive behavior may provide a useful procedure to search for new classes of antidepressants and also open possibilities to distinguish experimentally the molecular nature of sex differences in depression vulnerability. The effectiveness of BDNF after the administration of anisomycin in producing the antidepressant effect further raises the possibility that agents that enhance specifically the neurotrophic activity in the brain structures that control mood may be developed in the future as powerful antidepressants in both sexes.

References

- Abela, J.R.Z. (2001) The hopelessness theory of depression: A test of the diathesis-stress and causal mediation components in third and seventh grade children. *J Abnorm Child Psychol* **29**:241-254.
- Alonso SJ, Castellano MA and Rodriguez M (1991) Sex differences in behavioral despair: relationships between behavioral despair and open field activity. *Physiol Behav* **49**:69-72.
- Angst J, Gamma A, Gastpar M, Lepine JP, Mendlewicz J, Tylee A and Depression Research In European Society Study (2002) Gender differences in depression: Epidemiological findings from the European DEPRES I and II studies. *Eur Arch Psychiatry Clin Neurosci* **252**:201-219.
- Bai F, Li X, Clay M, Lindstrom T and Skolnick P (2001) Intr- and interstrain differences in models of “behavioral despair”. *Pharmacol Biochem Behav* **70**:187-192.
- Barros HMT and Ferigolo M (1998) Ethopharmacology of imipramine in the forced-swimming test: Gender differences. *Neurosci Biobehav Rev* **23**:279-286.
- Bebbington P (1996) The origins of sex differences in depressive disorder: bridging the gap. *Intern Rev Psychiatry* **8**:295-332.
- Bebbington P, Dunn G, Jenkins R, Lewis G, Brugha T, Farrell M and Meltzer H (2003) The influence of age and sex on the prevalence of depressive conditions: report from the National Survey of Psychiatric Morbidity. *Intern Rev Psychiatry* **15**:74-83.
- Burgess LH and Handa RJ (1992) Chronic estrogen-induced alterations in adrenocorticotropin and corticosterone secretion, and glucocorticoid receptor-mediated functions in female rats. *Endocrinology* **131**:1261-1269.
- Castrén E (2004) Neurotrophic effects of antidepressant drugs. *Curr Opin Pharmacol* **4**:58-64.

- Cavus I and Duman RS (2003) Influence of estradiol, stress, and 5-HT_{2A} agonist treatment on brain-derived neurotrophic factor expression in female rats. *Biol Psychiatry* **54**:59-69.
- Contreras CM, Lara-Morales H, Molina-Hernández M, Saavedra M and Arrelín-Rosas G (1995) An early lesion of the lateral septal nuclei produces changes in the forced swim test depending on gender. *Prog Neuropsychopharmacol Biol Psychiatry* **19**:1277-1284.
- Gibbs RB (1998) Levels of trkA and BDNF mRNA, but not NGF mRNA, fluctuate across the estrous cycle and increase in response to acute hormone replacement. *Brain Res* **787**:259-268.
- Handa RJ, Burgess LH, Kerr JE and O'Keefe JA (1994) Gonadal steroid hormone receptors and sex differences in the hypothalamo-pituitary-adrenal axis. *Horm Behav* **28**:464-476.
- Hattori E, Liu C, Zhu H and Gershon ES (2005) Genetic tests of biological systems in affective disorders. *Mol Psychiatry* **10**:719-740.
- Inda MC, Delgado-García JM and Carrión ÁM (2005) Acquisition, consolidation, reconsolidation, and extinction of eyelid conditioning responses require *de novo* protein synthesis. *J Neurosci* **25**:2070-2080.
- Kendler KS, Kuhn J and Prescott CA (2004) The relationship of neuroticism, sex, and stressful life events in the prediction of episodes of major depression. *Am J Psychiatry* **161**: 631-636.
- Kessler RC (2003): Epidemiology of women and depression. *J Affect Disord* **74**:5-13.
- Kitamura Y, Araki H and Gomita Y (2002) Influence of ACTH on the effects of imipramine, despramine and lithium on duration of immobility of rats in the forced swim test. *Pharmacol Biochem Behav* **71**:63-69.
- Klose M and Jacobi F (2004) Can gender differences in the prevalence of mental disorders be explained by sociodemographic factors? *Arch Womens Ment Health* **7**:133-148.

- Kornstein SG, Schatzberg AF, Thase ME, Yonkers KA, McCullough JP, Keitner GI, Gelenberg AJ, Ryan CE, Hess AL, Harrison W, Davis SM and Keller MB (2000) Gender differences in chronic major and double depression. *J Affect Disord* **60**:1-11.
- Krocicka B, Branski P, Palucha A, Pilc A and Nowak G (2001) Antidepressant-like properties of zinc in rodent forced swim test. *Brain Res Bull* **55**:297-300.
- Lattal KM and Abel T (2001) Different requirement for protein synthesis in acquisition and extinction of spatial preferences and context-evoked fear. *J Neurosci* **21**:5773-5780.
- Marvan ML, Chavez-Chavez L and Santana S (1996) Clomipramine modifies fluctuations of forced swimming immobility in different phases of the rat estrous cycle. *Arch Med Res* **27**:83-86.
- McEwen BS and Sapolsky RM (1995) Stress and cognitive function. *Curr Opin Neurobiol* **5**: 205-216.
- Miller WJ, Suzuki S, Miller LK, Handa R and Uht RM (2004) Estrogen receptor (ER) β isoforms rather than ER α regulate corticotropin-releasing hormone promoter activity through an alternate pathway. *J Neurosci* **24**:10628-10635.
- Nestler EJ, Barrot M, DiLeone RJ, Eisch AJ, Gold SJ and Monteggia LM (2002) Neurobiology of depression. *Neuron* **34**:13-25.
- Pang PT, Teng HK, Zaitsev E, Woo NT, Sakata K, Zhen S, Teng KK, Yung W-H, Hempstead BL and Lu B (2004) Cleavage of proBDNF by tPA/plasmin is essential for long-term hippocampal plasticity. *Science* **306**:487-491.
- Payne JL (2003) The role of estrogen in mood disorders in women. *Intern Rev Psychiatry* **15**:280-290.

- Piccinelli M and Wilkinson G (2000) Gender differences in depression: Critical review. *Br J Psychiatry* **177**:486-492.
- Rasika S, Alvarez-Buylla A and Nottebohm F (1999) BDNF mediates the effects of testosterone on the survival of new neurons in an adult brain. *Neuron* **22**:53-62.
- Rhodes ME, Kennell JS, Belz EE, Czambel RK and Rubin RT (2004) Rat estrous cycle influences the sexual diergism of HPA axis stimulation by nicotine. *Brain Res Bull* **64**:205-313.
- Russo-Neustadt AA, Beard RC, Huang YM and Cotman CW (2000) Physical activity and antidepressant treatment potentiate the expression of specific brain-derived neurotrophic factor transcripts in the rat hippocampus. *Neuroscience* **101**:305-312.
- Sairanen M, Lucas G, Ernfors P, Castrén M and Castrén E (2005) Brain-derived neurotrophic factor and antidepressant drugs have different but coordinated effects of neuronal turnover, proliferation, and survival in the adult dentate gyrus. *J Neurosci* **25**:1089-1094.
- Santarelli L, Saxe M, Gross C, Surget A, Battaglia F, Dulawa S, Weisstaub N, Lee J, Duman R, Arancio O, Belzung C and Hen R (2003) Requirement of hippocampal neurogenesis for the behavioral effects of antidepressants. *Science* **301**:805-809.
- Setnik B, de Souza FG, d'Almeida A and Nobrega JN (2004) Increased homocysteine levels associated with sex and stress in the learned helplessness model of depression. *Pharmacol Biochem Behav* **77**:155-161.
- Shirayama Y, Chen AC, Nakagawa S, Russell DS and Duman RS (2002) Brain-derived neurotrophic factor produces antidepressant effects in behavioral models of depression. *J Neurosci* **22**:3251-3261.

Steenbergen HL, Heinsbroek RPW, Van Haaren F and Van De Poll NE (1989) Sex-dependent effects of inescapable shock administration on behavior and subsequent escape performance in rats. *Physiol Behav* **45**:781-787.

Sun M-K and Alkon DL (2003) Open space swimming test to index antidepressant activity. *J Neurosci Meth* **126**:35-40.

Sun M-K and Alkon DL (2004) Induced depressive behavior impairs learning and memory in rats. *Neuroscience* **129**:129-139.

Sun M-K and Alkon DL (2005) Dual effects of bryostatin-1 on spatial memory and depression. *Eur J Pharmacol* **512**:43-51.

Takamori K, Yoshida S and Okuyama S (2001) Availability of learned helpless test as a model of depression compared to a forced swimming test in rats. *Pharmacology* **63**:147-153.

Viau V (2002) Functional cross-talk between the hypothalamic-pituitary-gonadal axes. *J Neuroendocrinol* **14**:506-513.

West AP (1990) Neurobehavioral studies of forced swimming: the role of learning and memory in the forced swim test. *Prog Neuropsychopharmacol Biol Psychiatry* **14**: 863-877.

Competing financial interests: declared none.

Figure Legends

Fig. 1. Sex differences in vulnerability to induction of depressive behavior and sensitivity to imipramine treatment. **a** and **b**, Mobility in the open space swim test (1 trial/day for 3 days) with the minutes/trial indicated of male (**a**) and female rats (**b**). Female rats were more sensitive than the males to the 11-min trials but the extent of the immobility induced by 15-min trials did not differ between the sexes. Arrows indicate effects of sex hormones (estradiol, **a**; androsterone, **b**). Data are mean \pm S. E. M. (8 rats per group). Imi: imipramine.

Fig. 2. Effects of induced depressive behavior and antidepressant treatment on depression-related memory impairment. **a** and **b**, Performance in a multi-trial passive avoidance task of male (**a**) and female rats (**b**), 24 hr after the third open space swim test trial (1 trial/day for 3 days). The number of the trials was the number of the training trials needed for the rats to learn to avoid the other compartment and punishment (un-escapable shock of 0.3 mA, 2s) for 120 s. **c** and **d**, Retention of the learned passive avoidance experience of male (**c**) and female rats (**d**), 24 hours after the task learning. The step-through latency was the time delay for the rats to enter the other compartment (1 trial without shock, cut-off 300 s). Data are mean \pm S. E. M. (8 rats per group). *: significant difference from the control. NS: no statistical difference from the control. Imi: imipramine. Control: rats that were not subject to the swim trials. No agents were administered during the multi-trial avoidance task.

Fig. 3. Vulnerability difference in female rats to induction of depressive behavior at different estrous cycle stages. Mobility in the short open space swim test (three 400 s trials at 2 h intervals between trials during the same day). Female rats were more sensitive than the males, in which

immobility was not induced. Proestrous and estrous females showed the highest vulnerability to the induction. Data are mean \pm SEM (8 rats per group).

Fig. 4. Sensitivity of open space swim test-induced immobility to mifepristone and spironolactone in male (15 minutes/trial, 1 trial/day for 3 days; **a**) and female rats (11 minutes/trial, 1 trial/day for 3 days; **b**). The immobility over trials was not induced in the rats in which mifepristone was administered, while spironolactone did not affect the induced immobility. Control: rats that were subject to 15 min/trial (1 trial/day) for 3 days (male) and 11 min/trial (1 trial/day) for 3 days (female). Data are mean \pm SEM (8 rats per group).

Fig. 5. Sensitivity of open space swim test-induced immobility to anisomycin and brain derived neurotrophic factor (BDNF) in male (11 minutes/trial, 1 trial/day for 3 days in the estradiol+anisomycin group and 15 minutes/trial, 1 trial/day for 3 days in the remaining groups; **a**) and female rats (11 minutes/trial, 1 trial/day for 3 days; **b**). The immobility over trials was not changed by anisomycin, which eliminated effects of imipramine and androsterone. Administration of BDNF effectively produced an antidepressive effect in rats in which anisomycin was administered. Imi: imipramine. Control: rats that were subject to 15 min/trial (1 trial/day) for 3 days (male) and 11 min/trial (1 trial/day) for 3 days (female). Data are mean \pm SEM (8 rats per group).

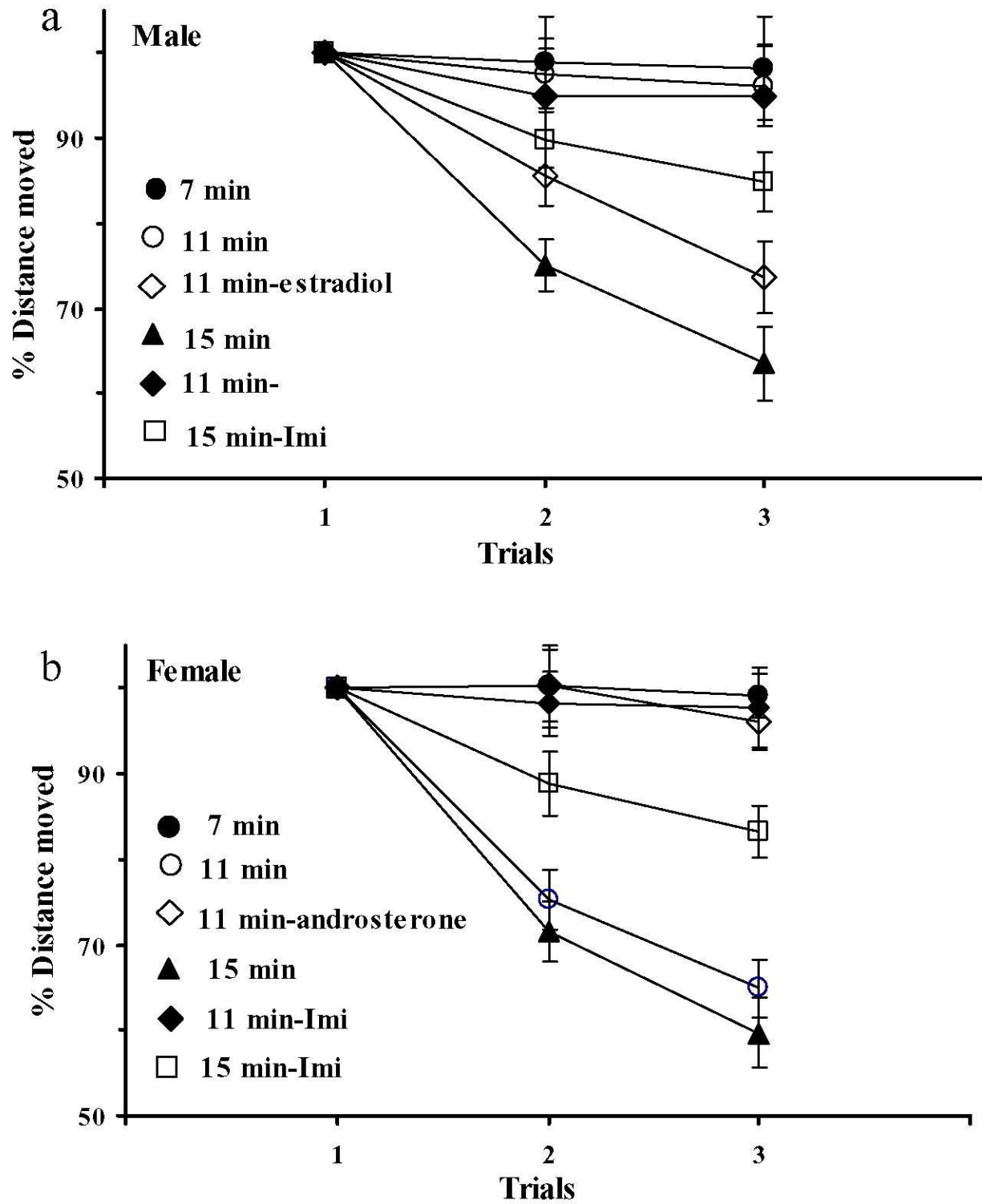


Figure 1

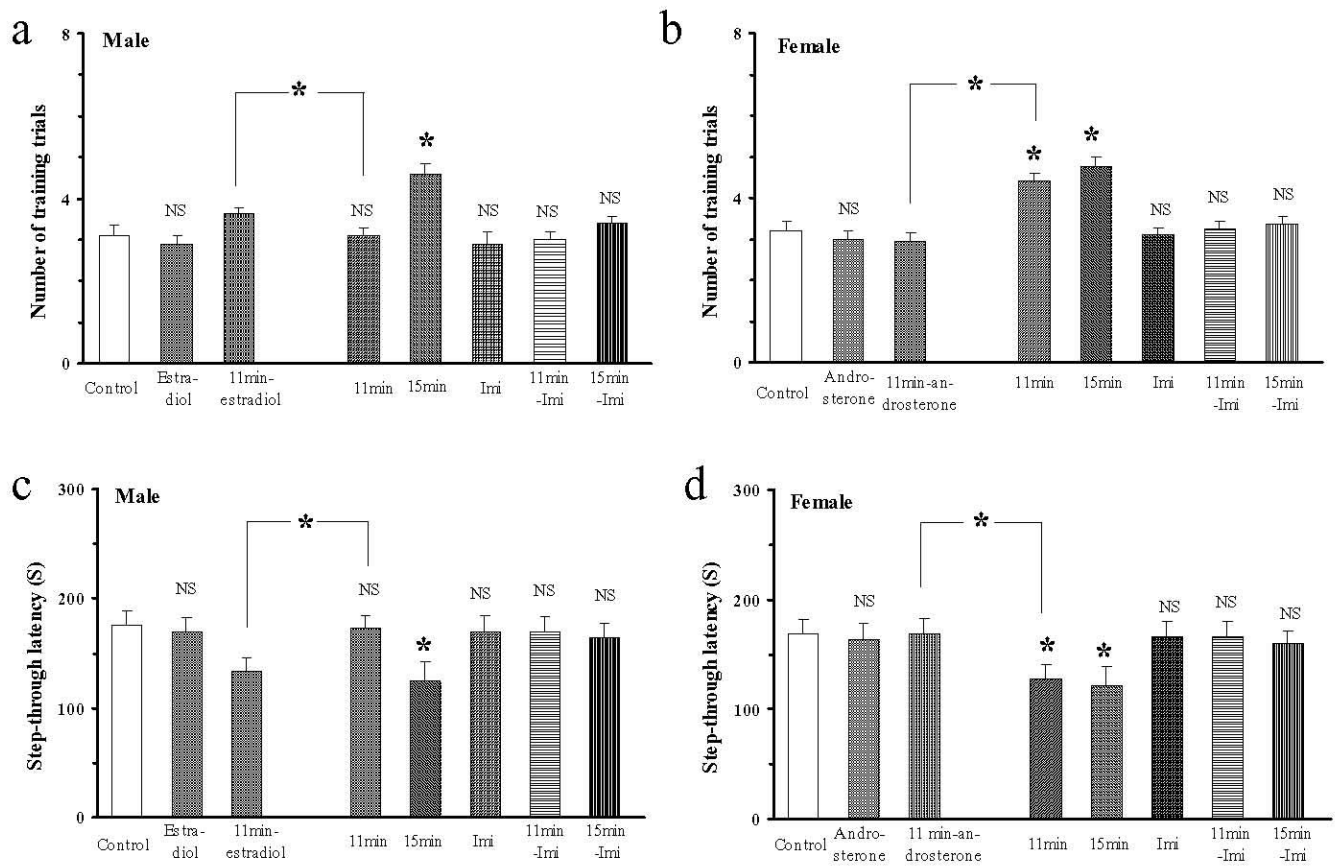


Figure 2

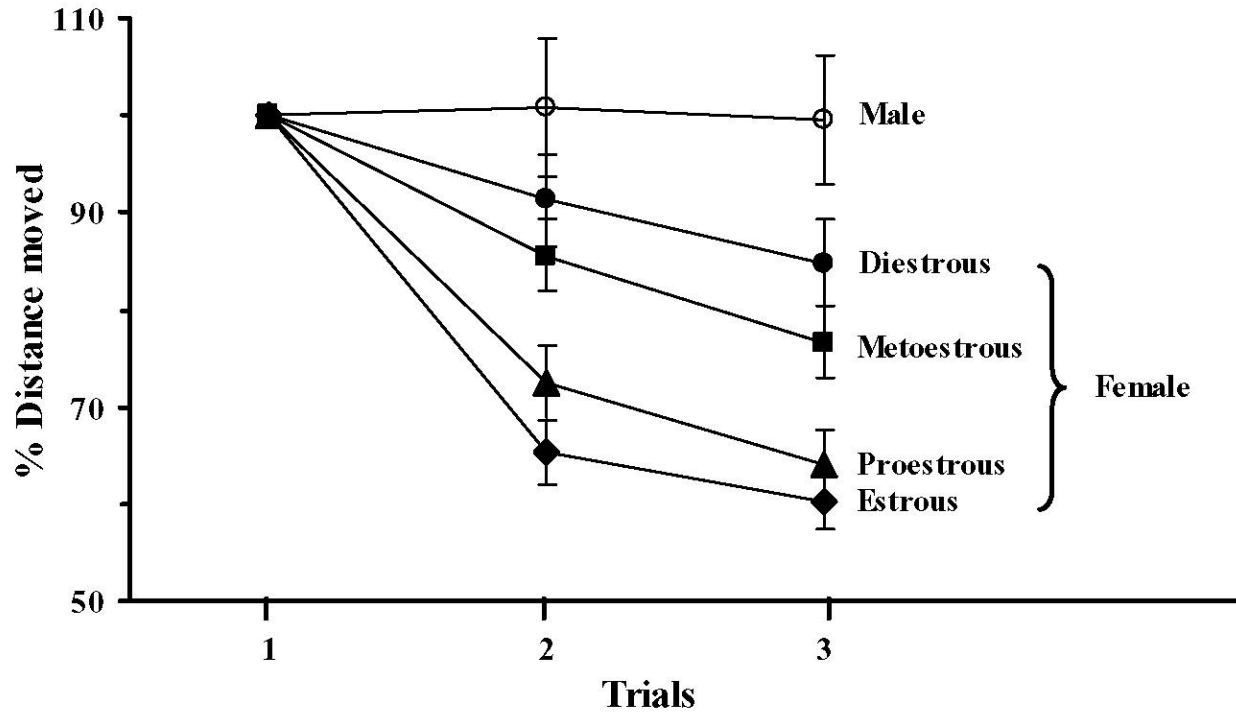


Figure 3

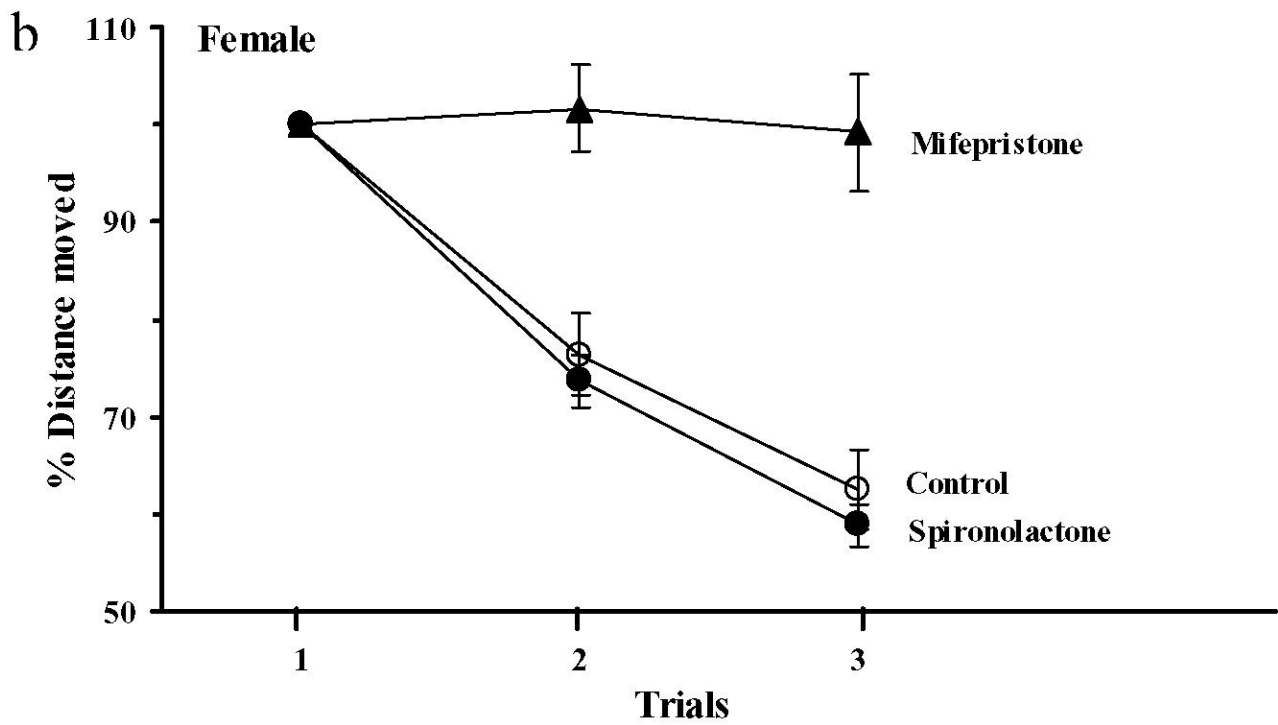
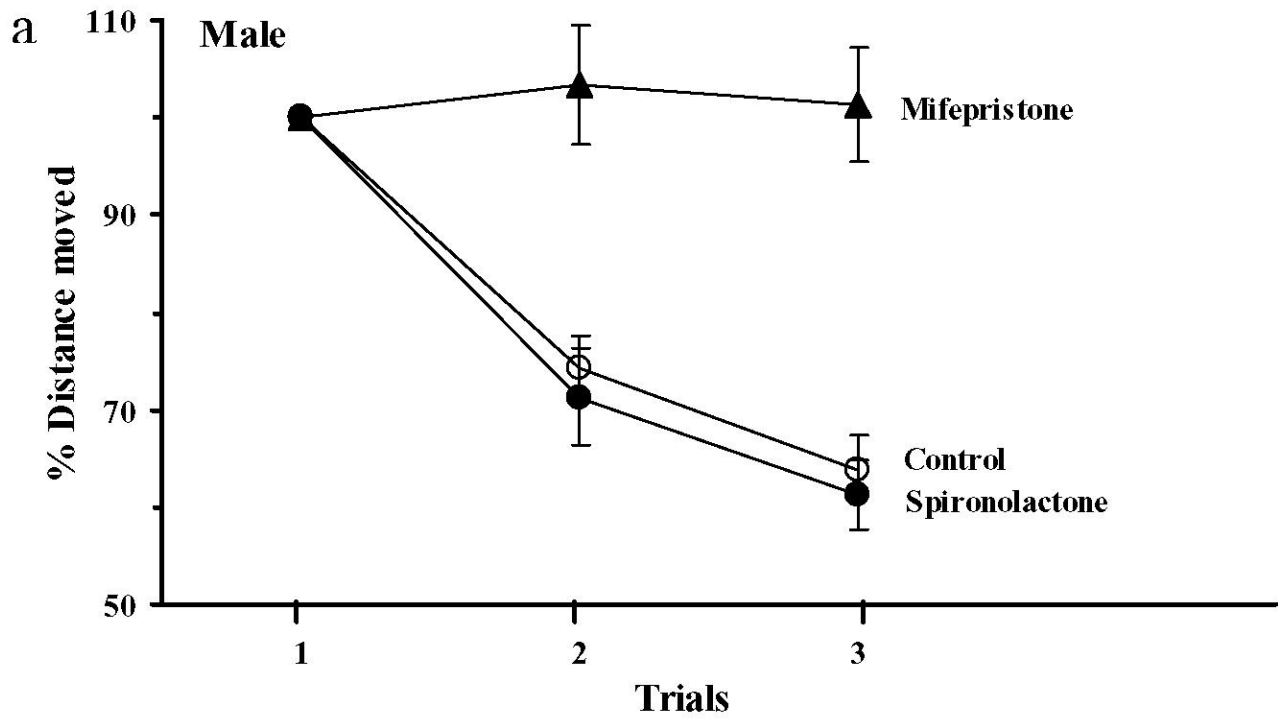


Figure 4

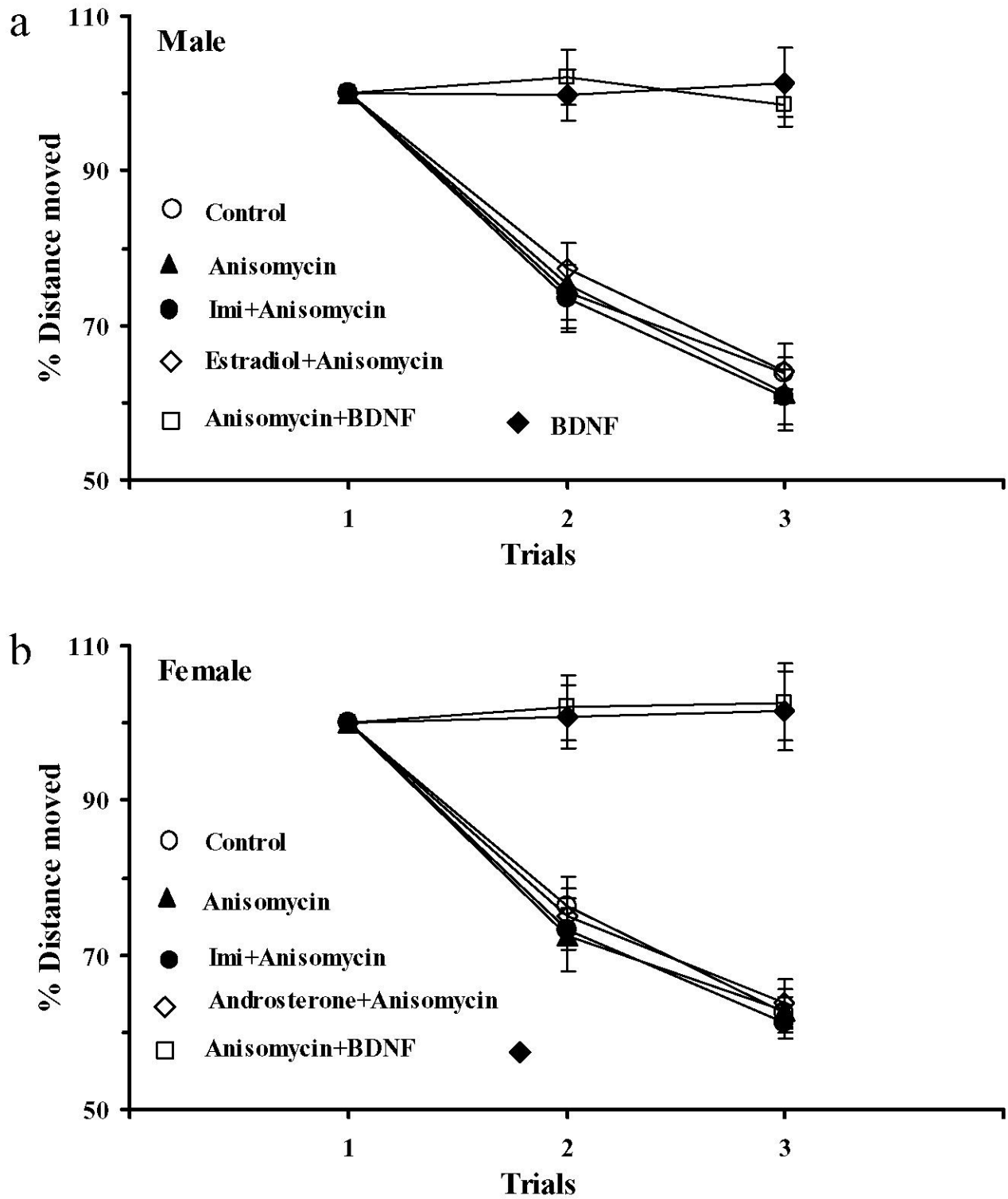


Figure 5