Journal of Experimental and Clinical Medicine
https://dergipark.org.tr/omujecm

Research Article

J Exp Clin Med
2022; 39(4): 1169-1174
doi: 10.52142/omujecm.39.4.42

Sex-related differences in anxiety in experimental functional dyspepsia induced by chronic stress

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Received: 20.06.2022 • **Accepted/Published Online:** 04.07.2022 • **Final Version:** 29.10.2022

Abstract

Functional dyspepsia (FD) is associated with gastric sensorimotor dysfunction (including delayed gastric emptying (GE)) and psychosocial comorbidities. Anxiety is among the many psychiatric disorders that are related to FD. The aim of this study was to compare the effectiveness of chronic sequential stress to create an experimental FD model in male and female rats. The FD model was established by neonatal maternal separation (MS) early in life and repeated homotypic stress (RHS) in adulthood. Newborn pups from postnatal day 1 to day-21 underwent MS for 3 h. In adulthood, the control or maternally separated rats were loaded with RHS for 7 days comprised of 90-min restraint stress. The anxiety-like behaviors were evaluated by the open field (OF) and elevated plus maze (EPM) tests. To validate the experimental FD model, body weight and solid GE were measured in rats after the behavioral experiments. Compared with control males, body weight and GE were significantly ($p<0.05$) decreased in stressed rats, but not in females. Exposed to chronic stress male rats appeared to exhibit more anxiety-like behavior than control male rats on the OF and EPM. In contrast to the males, no significant differences were found in female groups. Unlike female rats, the male rats appear to be highly suitable to create an experimental FD model under chronic sequential stressed conditions. Therefore, anxious behaviors may not be observed in females due to the absence of dyspeptic symptoms.

Keywords: anxiety, gender, functional dyspepsia, chronic stress

1. Introduction

Exposure to acute or chronic stress is a major risk factor for various functional gastrointestinal disorders (FGID) such as irritable bowel syndrome and functional dyspepsia (FD) (1-3). Adverse events in early life are one of the risk factors for developing FD in adulthood (4, 5). FD is characterized by upper gastrointestinal symptoms such as bloating, early postprandial satiety, epigastric pain, and burning (6, 7). Moreover, delayed gastric emptying (GE), body weight loss, and anxiety are considered to be pathophysiological features of FD (8, 9). The detection rate of anxiety symptoms in FD is 25.5% (10).

Recently, different experimental FD models have offered insights into understanding the FD process, its, pathology, etiology, and molecular mechanisms (11-13). The decision on which model should be chosen as a basis for researching FD varies depending on the study aims and questions of interest. Compound animal models with multiple factors contributing to the development of FD have come to the fore in recently published studies (14-17). It is thought that FD seen in adulthood may be caused by early stress exposure and re-exposure to stress later in life may aggravate the situation. Therefore, in this study, the experimental FD model was established by neonatal maternal separation (MS) early in life and repeated homotypic stress (RHS) in adulthood.

The purpose of this study is to examine sex-related differences in anxiety in chronically stressed rats. Especially, the suitability of the chronic sequential stress model, which is known to be effective in male rats, was tested for female rats.

2. Material and Methods

2.1. Animals

Adult male and female Wistar rats weighing 250-300 g were maintained on a 12 h:12 h dark-light cycle (starting at 6:00 AM) with access to food and water ad libitum. All experiments were performed in accordance with the Guide for the Care and Use of Laboratory Animals and approved by the Animal Ethical Committee of Akdeniz University (with unique authorization number B.30.2.AKD.0.05.07.00/138).

2.2. Chronic sequential stress model for functional dyspepsia (FD)

For the experimental FD model, chronic sequential stress was loaded with MS early in life and RHS in adulthood, as described elsewhere (14, 15). Newborn pups initially underwent neonatal MS for 3 h from postnatal day (PND)-1 to day-21. The control (non-stressed) pups were non-handled and kept with their dams. On PND 21, animals were weaned and

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sexed. Male control and MS pups were kept with their littermates until the experiments performed at their 12-week-old age. Adult MS rats were exposed to RHS comprised of 90-min restraint stress for 7 consecutive days. To confirm that body weight loss and delayed gastric emptying (GE) in our model, body weight was recorded and solid GE was measured in overnight fasted rats exposed to chronic stress or non-stressed animals after the behavioral experiments. The experimental procedures are schematically summarized in Fig.1.

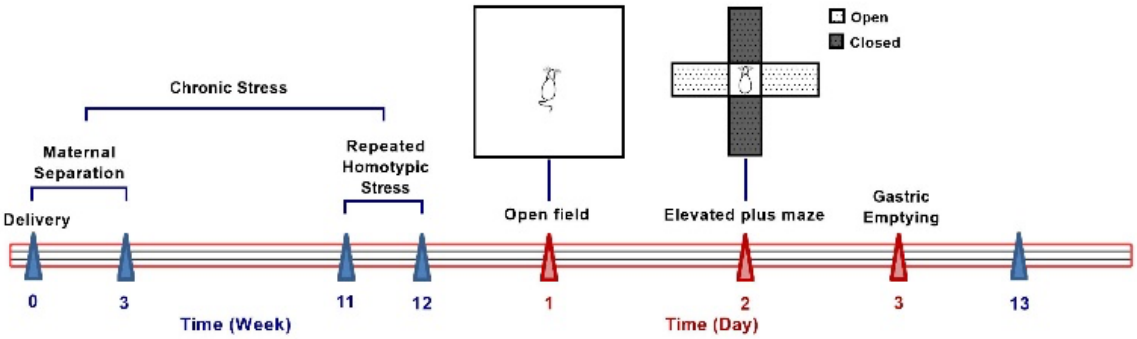

Fig. 1. Representative flow chart of the experimental design. The experiments were performed after the chronic stress loading

2.3. Measurement of gastric emptying (GE)

After the behavioral experiments, animals underwent overnight fasting, as previously reported (18, 19). Overnight fasting rats were given pre-weighed pellets (1.6 g). Immediately after the completion of feeding, rats were euthanized by urethane (1.25 g·kg-1, i.p) and the stomachs were removed and emptied thoroughly. The gastric contents were collected and dried at room temperature. After weighing, GE was calculated according to the following formula:

%GE=1-(weight of the dried content/weight of the pellet) ×100

2.4. Measurement of anxiety-like behaviors

The open field (OF) test and elevated plus maze (EPM) test were used to FD-induced anxiety-related behaviors. The OF and EPM were performed as described in previous studies (20-22). Briefly, the rats were gently placed in the center of an open field chamber. A more anxious animal would present more time at the periphery than in the center. For EPM, each animal was placed onto the central platform facing an open arm. The increase in open arm activity reflects less anxiety-like behavior. All rats were allowed to freely explore the maze for 5 min. Video-tracking system (Ethovision XT, Noldus Information Technology, Netherlands) was used to record and analyze the behavioral tests. The anxiety-related behaviors were evaluated by measuring the time spent in closed/open arms (s), the number of open arm entries, time in center/periphery (s), and entries in the center. An increase in periphery or close arm duration reflects anxiety-related behavior. All areas were cleaned with 70% ethanol solution after each test.

2.5. Statistics

All statistical analyses were performed using Prism 9 software (GraphPad Software, Inc, La Jolla, CA). All data are presented as mean ± standard error of the mean (SEM). Shapiro-Wilk test was used to determine whether the data were normally distributed. The non-parametric Mann Whitney-U test was used to assess the significance. A p value <0.05 was considered as statistically significant.

3. Results

3.1. Validation of functional dyspepsia model

In order to validate the experimental FD model, body weight and solid GE were measured. The body weight of male rats exposed to chronic stress was significantly decreased (258.6±15.3 g, $p<0.05$, n=6) compared with the control male rats (341.4±5.3 g, n=6). Unlike males, chronic stress did not affect the body weight of female rats (194±5.4 g, n=6) compared to that in control females (188.3±6.2 g, n=6), (Fig. 2A)

The measured GE in control male rats was 66.2±5.6 (n=6), however, it was significantly delayed in male rats exposed to chronic stress (37.6±5, $p<0.05$, n=6). In contrast to the males, chronic stress had no significant effect on females (control: 54.1±4.2%; chronic stress: 60.2±5.4%, n=6), (Fig. 2B)

Taken together, these results suggest that the experimental FD model was established successfully in male rats, however, it is ineffective in female rats.

3.2. Sex-related differences in anxiety

To investigate the effect of chronic stress on anxiety-like behavior, we performed the OF and EPM tests. Exposed to chronic stress male rats appeared to exhibit more anxiety-like behavior than control male rats on the OF, as indicated by greater total time spent in the periphery (control: 283.7±2 s; chronic stress: 293.2±2.3 s, $p<0.05$, n=6) and lower spent in center (control: 11.7±0.9 s; chronic stress: 1.2±0.3 s, $p<0.05$, n=6) of the maze. Similarly, the number of entries to the center zone of the OF was also significantly less in stressed male rats (5.3±0.4, $p<0.05$, n=6) compared to control males (15.4±2.2,

n=6), but not in females. Unlike males, no differences were found in time spent in the center/periphery of the OF between the stressed and non-stressed female groups (Fig.3).

In the EPM test, chronic stress-treated male rats spent more time in closed arms (254.9±16.8 s, $p<0.05$, n=6) than control counterparts (165.9±27 s, n=6), and stressed males also spent less time in open arms (230±4.9 s, $p<0.01$, n=6) than non-stressed males (114.2±13.2 s, n=6), which are the common indexes of anxiety-like behavior. In contrast to the males, no differences were found in time spent in open/closed arms and the number of open arms entries between female groups (Fig.4).

These results suggest that chronic stressed male rats demonstrated typical anxiety-like behaviors, but not in females.

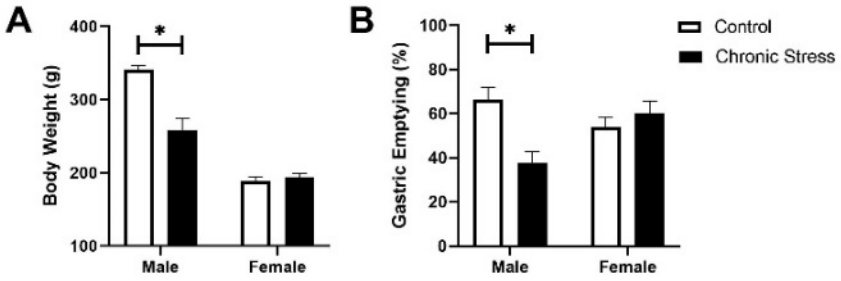

Fig. 2. The effect of chronic stress on body weight (A) and gastric emptying (B) in male and female rats. Chronic stress decreased body weight and gastric emptying in males. *p<0.05, control vs chronic stress. Mann Whitney-U test was used to carry out statistical comparisons, n=6 rats per group. All values are means ± SEM

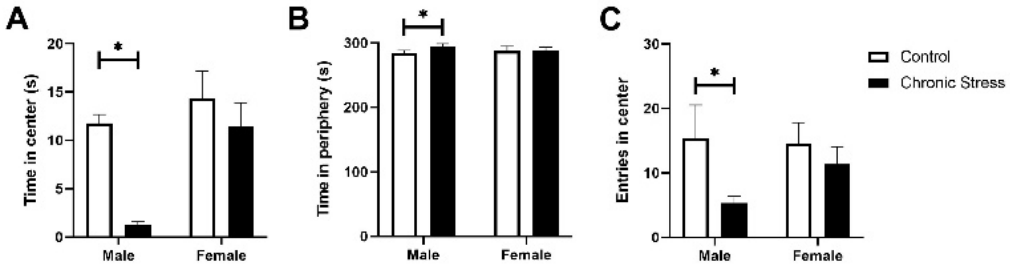

Fig. 3. The effect of chronic stress on the behavior of male and female rats in the open field test. Chronic stress increased anxiety-related behaviors in males. *p<0.05, control vs chronic stress. Mann Whitney-U test was used to carry out statistical comparisons, n=6 rats per group. All values are means ± SEM

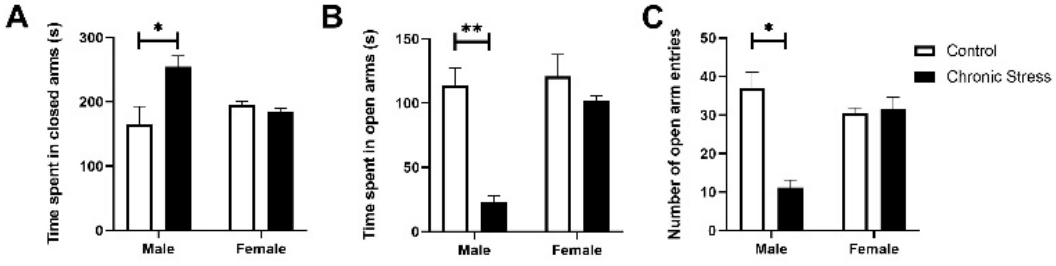

Fig. 4. The effect of chronic stress on the behavior of male and female rats in the elevated plus maze. Chronic stress increased anxiety-related behaviors in males. *p<0.05, **p<0.01, control vs chronic stress. Mann Whitney-U test was used to carry out statistical comparisons, n=6 rats per group. All values are means ± SEM

4. Discussion

The findings of this study indicate that exposure to chronic sequential stress could lead to an experimental FD model with decreased body weight, GE, and increased anxiety-like behavior in male rats, but not in females. A summary of the all results is shown in Fig.5.

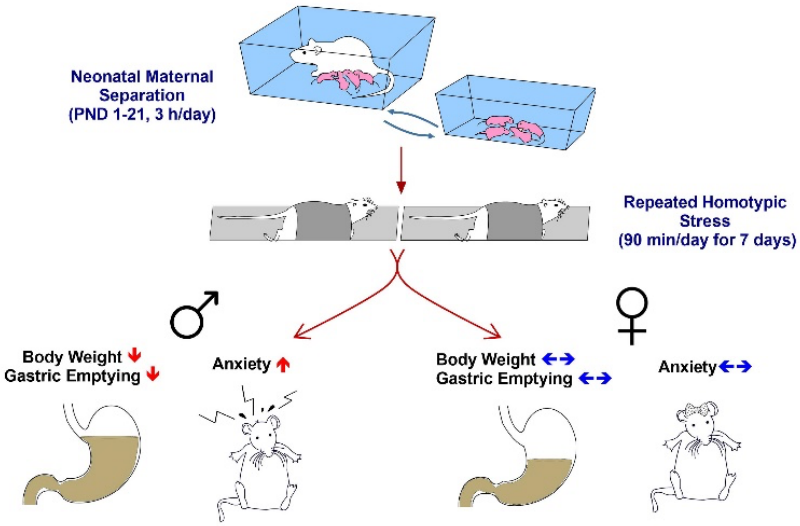

Fig. 5. A schematic summary of the results.

Stress contributes to the onset and exacerbation of symptoms in the majority of FGID (1). Thus, the different stress models are widely used as an experiment for the investigation of FGID or FD (14-16). In recent preclinical studies, a compound animal model that includes two or more stressors has been preferred for FD development (14-17). However, no sex differences have also been reported in these studies. Thus, the efficacy of this compound model in female rats is still unknown. Consequently, in this study, the experimental FD model was established by neonatal MS followed by adult RHS in male and female rats to observe sex-related differences in anxiety.

Pathophysiologic mechanisms of FD include slowed gastric emptying, impaired gastric accommodation, visceral hypersensitivity, duodenal inflammation, and psychosocial factors (6, 23). Evidence from epidemiologic studies suggests dyspeptic patients experience early satiety and weight loss due to gastric accommodation disorder (9, 24). Thus, in this study, body weight and GE were measured to validate the experimental FD model. Our present data showed that the body weight decreased only in chronically stressed male rats compared to non-stressed rats. In rodent studies, FD-induced delayed GE has been found in several measures (15, 25, 26). In this study, dyspeptic symptoms expected to occur in animals in the FD group were evaluated by the measurement of solid GE. Similar to previous studies, delayed GE has been detected in male stressed rats. On the other hand, no significant effect of chronic stress was observed in females. These results suggest that the experimental FD model was established successfully in male rats as previously reported in studies using chronic sequential stress, however, it is ineffective in female rats. Importantly, it was shown that the acute stress-induced gastrointestinal motor dysfunction were completely recovered following 5-day RHS loading (27, 28). In response to a chronic sequential stressor, adaptation may have occurred in female rats.

Anxiety, one of the most common symptoms among FD patients, has a high (>30%) incidence clinically (29). In this study, anxiety was evaluated with EPM and OF tests, which are frequently used in the literature to assess anxiety-related behaviors (30, 31). Consistent with the literature, exposed to chronic sequential stress male rats presented distinctive anxiety-like behavior, but not in females. Considering that even MS alone causes anxiety-like behaviors (32, 33), possible adaptation mechanisms in female rats due to RHS-loading may be in question. The discrepancies may also relate to the estrous cycle. Multiple studies have demonstrated the anxiolytic effects of both estradiol and progesterone (34-36). Future experiments about gender differences in stress responses are needed to confirm such a possibility.

The efficacy of this chronic sequential stress model in female Wistar rats is unknown. The effectiveness of this chronic sequential stress on female rats was first demonstrated in this study. Taken together, the male Wistar rats are more suitable to create an experimental FD model under chronic sequential stressed conditions compared to the females. It is important to note that the males seem to be vulnerable to chronic sequential stress.

Conflict of interest

No conflicts of interest exist

Funding

None to declare.

Acknowledgments

None to declare.

Authors' contributions

Concept: O.S., Design: O.S., Data Collection or Processing: O.S., Analysis or Interpretation: O.S., Literature Search: O.S., Writing: O.S.

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