GENDER DIFFERENCE ON BEHAVIORAL CHANGES AFTER COLD STRESS IN WISTAR ALBINO RATS

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ABSTRACT

Aim of the study: To determine the effect of acute and chronic cold water swimming stress on male and female Wistar albino rats.

Materials and methods: This study was done in department of Physiology, MMCH&RI, Kanchipuram. 36 Wistar albino rats of both sexes were divided into six groups with six animals in each group. Group I & II control group male & female, group III & IV acute cold stress male and female, group V & VI chronic cold stress male and female respectively. Stress animals were subjected to cold stress by placing animals at 10°C until it sinks. After some interval animals were subjected to behavioral assessment by using standardized models as Elevated Plus Maze (EPM) and Open Field Maze (OPM).

Results: Statistical analysis of behavioral assessment showed significant changes in both acute and chronic cold stressed animals. In open field data showed significant increase in immobilization time (P< 0.05) accompanied with significant decrease in no. of rearing (P< 0.05) grooming (P< 0.05) and ambulation behavior both in peripheral (P< 0.05) and central squares in both male and female rats of all groups. In elevated plus maze there was a significant increase in transfer latency duration (P< 0.05) with closed arm duration (P< 0.05) significant decrease in open arm duration (P< 0.05 ) and number of times arms crossed (P< 0.05) in both male and female rats, but comparatively the female rats showed high significance of behavioral changes when compared to male rats. Simultaneously the group subjected to chronic cold stress showed more stressor level than acute group.

Conclusion: This study concluded that female rats exposed to chronic stress showed high stressor effect than acute and male rats on behavioral changes.

Key Words: Gender difference, Cold stress, Elevated plus maze (EPM), Open field maze (OPM).

INTRODUCTION

Stress is a common factor observed long back and become popular in recent era. Stress affects almost all the systems of the body especially both behavior and physiology¹. Stressful conditions stimulate the hypothalamo pituitary adrenal (HPA) axis² which in turn release the corticotrophin releasing hormone (CRH) which cause release of adrenocorticotropic (ACTH) from anterior pituitary that further stimulate the secretion of glucocorticoids from the adrenal cortex³,⁴. Stress alters homeostasis which involved in the pathogenesis of number of diseases like gastric ulcer⁴, diabetes⁶, hypertension, heart diseases⁷, immuno-suppression⁸, mental depression⁹ etc.

The increased level of glucocorticoids is a prime marker of stress. Exposure to changes in temperature may alter the homeostasis. The changes in temperature might be hot or cold environment, when exposed extremely produces stress. The changes in temperature especially extreme cold affect negatively the performance and behavior of humans¹⁰. Extreme Cold exposure impairs motor performance¹¹,¹², cognition¹³,¹⁴, muscle endurance¹⁵ etc.

The effect of stress altered due to duration like acute or chronic which might affects the homeostasis. The changes resulted after stress may vary depends upon the duration of the stress¹⁶.This leads to various changes in HPA axis reflected in changes in neuroendocrine system of the body like...
increased corticosterone\textsuperscript{17}, increased free radicals\textsuperscript{18}, altered behavior\textsuperscript{19}, psychological changes\textsuperscript{20} etc.,

Behavioral changes are the important assessment of stress in recent times which found to be important marker of stress. The behavior was assessed by standardized behavioral models. Different animal models for stress have been developed recently and used frequently to evaluate the stress effect. The behavior models like Open Field maze, Elevated plus Maze will implement the effective changes after exposure to stress\textsuperscript{21}. These behavioral models assess the changes involving general loco motor activity and exploratory behavior.

Animals exposed to acute or chronic stress might be altered according to the type of exposure. There was evidence that males and females respond differently to stress, which determined genetically. This dimorphic change in behavior is due to influence of genes, gonads, sexual hormones, influence on brain, social integration etc.\textsuperscript{22}. The temperature changes especially cold also show sexual dimorphism. The cognitive function and voluntary motor function varies between male and female after exposure to stress. The aim of the present study is to investigate whether the cold stress shows sexual dimorphism in both and female rats. The rats exposed to cold stress of both acute and chronic type does influence the behavioral changes using standardized behavioral models.

**MATERIALS AND METHODS**

The experiment was carried out in the department of Physiology, Meenakshi Medical College Hospital & Research Institute, Enathur, Kanchipuram. The approval of Institutional Animal Ethical committee (Ref no: KN/COL/3410/2014) and care of experimental animals was taken as per CPCSEA guidelines.

Wistar albino rats of both sex weighing 200 -300g were used for the study. The total number of animals used was 36 which was divided into 6 groups with 6 animals each group. Group I & II divided as control group for male & female, group III & IV as acute cold stress for male and female, group V & VI as chronic cold stress for male and female rats respectively.

Group (III, IV, V, and VI) rats were subjected to both acute & chronic cold water swimming stress. Control group rats were left free in home cage with free access to food and water test baseline data in the same environment were stress to other group to be performed.

**Cold stress**

The cold stress test was conducted according to the method of Porsolt et al.\textsuperscript{(1978)} the rats were forced to swim in a fresh water at 10°c were introduced into the container. Initially the response will be vigorous swimming and after few minutes their activity begin to subside and eventually they ceased to move and float an upright position making only small movements to keep their heads above the water. After some time the rat began to sink down then rat was taken out of water wiped with dry cloth and protected. After 20 minutes of standard recovery time the albino rats were subjected to behavioural studies.

**Behavioral Assessment**

The changes in behaviour of rats following stress were evaluated by open field maze and elevated plus maze method.

**Open Field Maze**

This is the classical model of loco-motor and exploratory activities (Bhattacharya and Satyan, 1997 and Takayoshi et.al, 2006). The apparatus for the open field test is a square enclosure made of wood. The field was a closed area which is divided into 25 spaces equally. The 100 W frosted bulb was placed above the field during the activity testing. The behavioral parameters of each rat were tested in a wake condition in open field Maze for 3 minutes.

Testing was carried out in a temperature, noise and light controlled room. During the test procedure silence was maintained in the test room. The rats were placed in a cage in the testing room an hour before the test in order for them to aclimatize to the new environment\textsuperscript{31}. The open field was cleaned with 70% ethanol after each rat had been tested individually. Throughout the entire testing-session, the sequence of events and procedures should always be the same and the test circumstances (handling, room-features, equipment used) were all standardized and controlled as possible.

To analyze exploratory and loco motor activities as an indication of stress in the rat, animals were placed in the left rear quadrant of an open field. The number of line crossings and the total distance covered by the rat were measured. The more time the rat spends in the inner zone of the open field maze, and the more exploratory the rat is, the less stressed it is perceived to be\textsuperscript{32}.

I) **Immobilization Time:** It is the duration of time the rats were holding its head against the gravity but without movements of head, body or limb with opened eyes.

II) **Grooming:** Rhythmic paw movement over the face or head for face rubbing includes episodes of biting and cleaning of paws.

III) **Rearing:** Standing still on upright on its hind limb only.

IV) **Ambulation:** When all the four limbs were in one particular square (central or peripheral) of the open field maze.

**Elevated Plus Maze**

The maze had two open arms (50 cm X 10 cm) at right angle to it, two crossed arms (50 cm X 10 cm X 40 cm) with the
The percentage of total roof uncovered an open central crossing (10cm X 10 cm) and was rising to a height of 50 cm from floor. The behavioral parameters of each rat were tested for 5 minutes in wake condition in Elevated Plus Maze by placing them at the end of an open arm are:

i) Transfer latency: Time taken by the animal to move from the outer end of the open arm to either of two closed arm

ii) Percentage time in open arm: The percentage of total testing time spent in the open arm

iii) Percentage time in closed arm: The percentage of total testing time spent in the closed arm

iv) Number of crossing of the arms: The number of times the animal crosses the center for going one arm to any other of three arms.

**RESULTS**

Statistical analysis was done by using statistical software package SPSS Windows Version 14.0. The results were expressed as Mean±SD if the variable were continuous. The two tailed student ‘t’ test was used for comparing control, acute and chronic stress groups.

The P value <0.05 was considered statistically significant.

The data of behavioral analysis following acute and chronic stress with respect to control group analyzed is given in the table below. Exposure to Cold water swimming stress showed significant changes in animal behavior in both open field maze and elevated plus maze. In open field maze (Table, 1 & 2) there was a significant increase in immobilization time (P< 0.05) accompanied with significant decrease in no. of rearing (P< 0.05) grooming (P< 0.05) and ambulation behavior such as peripheral squares (P< 0.05) and central squares observed in both male and female rats. Comparatively in this study the values of behavior showed a highly significant level in female rats when compared to male rats. Simultaneously rats subjected to chronic cold stress showed significantly most stressor values than acute cold stress rats.

In elevated plus maze (Table 3 & 4) there was a significant increase in transfer latency (P< 0.05) with time spent in closed arms (P< 0.05) and decrease in time spent in open arms (P<0.05) and number of arms crossed (P< 0.05) in both male and female rats but comparatively the female rats showed a highly significant level when compared to male rats. Simultaneously, rats subjected to chronic cold stress showed a highly stressor level of behavior changes than acute cold stress rats.

**DISCUSSION**

Rats were a valid model to understand the extent to which gender and genotypic differences in stress sensitivity are biologically based rather than socially or culturally determined. Cold stress proved as a potent stressor that might be due to increased level of corticosterone, and free radicals.

Rats exposed to cold stress in both acute & chronic group showed the stress effect with alteration in behaviour might be due to increased level of free radicals which highly reactive moieties are playing an important role in health and disease. The brain is especially vulnerable to free radical damage because of its high oxygen consumption, abundant lipid content and relative paucity of antioxidant enzymes compared with other tissues.

The behavioural changes observed in OFM showed significant changes in the behavior like exploration and ambulation. There was significant decrease in rearing, grooming, and central ambulation with simultaneous significant increase in immobilization time in female rats compared to male rats. This might be due to release of vasopressin which is stimulated during stress. However in female rats a small but significant rise in plasma vasopressin levels was observed. Gender differences in hypothalamic vasopressin mRNA levels and plasma osmolality were described indicating higher values in females.

The behavior changes observed in EPM showed significant increase in transfer latency, closed arm time, with simultaneously decreases in open arm time, number of crossings. The changes above mention was significantly more in females than male rats which could be due to increased release of oxytocin, an important component of the neuroendocrine response to the majority of stress stimuli. Simultaneously, the oxytocin release could be added by the secretion from HPA axis which might be important stimuli for more stress in females than males.

Behavioral alternation in rats was higher in female rats it may be due to the higher levels of corticosteroid binding globulin in females, which result in similar free circulating levels of corticosterone in the both sex, but the corticosterone level in brain appears to be similar in the both sex before stress.

In EPM, there was significant increase in closed arm time in chronic group when compared to acute group that could be due to increased level of corticosterone as repeated stress maintains high levels continuously than acute group which could be due to repeated exposure to stressor which is necessary to alter behaviour and neuro endocrine response.

Similarly in OFM, the rats showed significant increases in immobilization time that might be due to impaired antioxidant level in the brain tissue presumably through production of excessive reactive oxygen species, the end product of excess stress. The effect of stress expressed as behavioral changes were found to be more significantly in chronic groups than rats of acute group.
CONCLUSION

In this study, the gender difference was highly appreciated by behavioral changes observed in EPM and OFM after cold water swimming stress. The rats exposed to repeated or chronic stress showed a high stressor effect than exposed to acute stress. Female rats when compared to their counterpart showed more stressor effect and the groups exposed to chronic stress had higher stressor effects than acute groups. Hence, the gender difference and duration of stress influences more on behavioral changes after chronic cold stress.

REFERENCES


Table 1: Effect of cold stress on Male rats-OFM

<table>
<thead>
<tr>
<th>Behavioural parameters</th>
<th>Control</th>
<th>Acute cold Stress</th>
<th>P value</th>
<th>Chronic cold stress</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rearing (No.)</td>
<td>39±4</td>
<td>27±6</td>
<td>0.001*</td>
<td>17.5 ± 4</td>
<td>0.000*</td>
</tr>
<tr>
<td>Grooming(No.)</td>
<td>27±4</td>
<td>23±4</td>
<td>0.001*</td>
<td>23.5 ± 5</td>
<td>0.001*</td>
</tr>
<tr>
<td>Peripheral Square (No.)</td>
<td>36±5</td>
<td>23±3</td>
<td>0.0003*</td>
<td>30 ± 4</td>
<td>0.044*</td>
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<tr>
<td>Central Square (No.)</td>
<td>8.7 ± 3</td>
<td>6±2</td>
<td>0.001*</td>
<td>4 ± 2</td>
<td>0.004*</td>
</tr>
<tr>
<td>Immobilization time (sec)</td>
<td>17 ± 3</td>
<td>25±8</td>
<td>0.0447*</td>
<td>29±9</td>
<td>0.0113*</td>
</tr>
</tbody>
</table>

* Significant value (P<0.05)

Table 2: Effect of cold stress on Female rats-OFM

<table>
<thead>
<tr>
<th>Behavioural parameters</th>
<th>Control</th>
<th>Acute cold Stress</th>
<th>P value</th>
<th>Chronic cold stress</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rearing (No.)</td>
<td>34±5</td>
<td>6±2</td>
<td>0.0001*</td>
<td>16 ± 5</td>
<td>0.0001*</td>
</tr>
<tr>
<td>Grooming(No.)</td>
<td>67±7</td>
<td>31±5</td>
<td>0.0001*</td>
<td>24 ± 7</td>
<td>0.0001*</td>
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<tr>
<td>Peripheral Square (No.)</td>
<td>65.2±5</td>
<td>37.9 ± 5</td>
<td>0.001*</td>
<td>28.7 ± 5.8</td>
<td>0.000*</td>
</tr>
<tr>
<td>Central Square (No.)</td>
<td>12 ± 3</td>
<td>7±2</td>
<td>0.0068*</td>
<td>4 ± 2</td>
<td>0.0003*</td>
</tr>
<tr>
<td>Immobilization time (sec)</td>
<td>16 ± 4</td>
<td>31±3</td>
<td>0.0001*</td>
<td>28±7</td>
<td>0.0045*</td>
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</table>

* Significant value (P<0.05)

Table 3: Effect of cold stress on Male rats Elevated plus maze

<table>
<thead>
<tr>
<th>Behavioural parameters</th>
<th>Control</th>
<th>Acute</th>
<th>P value</th>
<th>Chronic</th>
<th>P value</th>
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</thead>
<tbody>
<tr>
<td>Transfer latency (sec)</td>
<td>16±5</td>
<td>25±4</td>
<td>0.0063*</td>
<td>67±11</td>
<td>0.0001*</td>
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<tr>
<td>Open arm (sec)</td>
<td>140±10</td>
<td>31±7</td>
<td>0.0001*</td>
<td>65±8</td>
<td>0.0001*</td>
</tr>
<tr>
<td>Closed arm (sec)</td>
<td>160±10</td>
<td>232±7</td>
<td>0.0001*</td>
<td>269±36</td>
<td>0.0001*</td>
</tr>
<tr>
<td>Number of crossings</td>
<td>7±2</td>
<td>1±0.6</td>
<td>0.0001*</td>
<td>1.3±0.8</td>
<td>0.0001*</td>
</tr>
</tbody>
</table>

*Significant value (P<0.05)
Table 4: Effect of cold stress on Female rats Elevated plus maze

<table>
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<tr>
<th>Behavioural parameters</th>
<th>Control</th>
<th>Acute</th>
<th>P value</th>
<th>Chronic</th>
<th>P value</th>
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</thead>
<tbody>
<tr>
<td>Transfer latency (sec)</td>
<td>18±5</td>
<td>26±3</td>
<td>0.0072*</td>
<td>32±5</td>
<td>0.0007*</td>
</tr>
<tr>
<td>Open arm (sec)</td>
<td>148±10</td>
<td>68±11</td>
<td>0.0001*</td>
<td>33±4</td>
<td>0.0001*</td>
</tr>
<tr>
<td>Closed arm (sec)</td>
<td>167±10</td>
<td>269±11</td>
<td>0.0001*</td>
<td>237±6</td>
<td>0.0001*</td>
</tr>
<tr>
<td>Number of crossings</td>
<td>8±2</td>
<td>2±0.7</td>
<td>0.0002*</td>
<td>3±0.5</td>
<td>0.0008*</td>
</tr>
</tbody>
</table>

* Significant value (P<0.05)