International Journal of Medical, Medicine and Health Sciences
ISSN: 2517-9969
Vol:9, No:2, 2015

# Study Regarding Effect of Isolation on Social Behaviour in Mice

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***Abstract*****—**Humans are social mammals, of the primate order. Our biology, our behaviour and our pathologies are unique to us. In our desire to understand, reduce solitary confinement one source of information is the many reports of social isolation of other social mammals, especially primates. A behavioural study was conducted in the department of pharmacology at Indira Gandhi Medical College, Shimla in Himachalpradesh province in India using white albino mice. Different behavioural parameters were observed by using open field, tail suspension, tests for aggressive behaviour and social interactions and the effect of isolation was studied. The results were evaluated and the standard statistics were applied. The said study was done to establish facts that isolation itself impairs social behaviour and can lead to alcohol dependence as well as related drug dependence.

***Keywords*****—**Albino Mice, Drug Dependence, Social isolation.

## I. Introduction

HUMANS are social mammals. For understanding behavioral aspects of humans residing in solitary confinement environmental conditions were studied. Environmental conditions that experimental animals are exposed to have been thought to affect various aspects of their behavior [1]. Previous studies have reported that enriched environments can improve experimental animals' cognitive functions, causing abnormal behaviours. There are studies which show that socially isolated animals are more depressed and more anxious than animals which are grouped together [2]. There are studies where it has been studied that a significant increase of locomotors activity is present in isolated mice [3]. Studies results have also linked social isolation to more aggressive behavior [4]. There are also studies showing that social isolation selectively elevates animal anxiety without affecting depression like behaviours [5].

Here we have examined the affects of isolation in mice on anxiety, aggressiveness, social interactions and hence demonstrated the specific impact of social isolation on emotional behaviour.

### *A. Methods*

1. Animals and Rearing Environments

The mice used in this study were white, albino mice. The temperature for rearing the mice and further behavioural environment was 18 to 20 degree Celsius. Humidity was 68 %. The study was done in the day time from 9 am to 6 pm. The cages used for the experimental study were of the size 40 cms x 26 cms x 15 cms.

2. Open Field Test and Analysis

The open field test was carried out in an acrylic container and the starting position within the container was same for all mice. Subjects open field exposure time was 15 mins. Time was measured in seconds that each subjects spent in the central 20x 20 cms area of the open field.

3. Test for Aggressive Behaviour

The cages used for the experimental study were of the size 40 cmsx26x15 cms. The subjects were kept in individual isolation for 6 weeks and after that they were housed together. The control group was also studied.

4. Test for Social Interactions in Mice*:*

The cages used for the experimental study were of the size 40 cmsx26x15 cms. The subjects were kept in individual isolation for 6 weeks and after that they were housed together. The control group was also studied.

5. Tail Suspension Test

A steel bar was used for the test. The distance between floor and tail was about 30 cms and the observation time was 6 mins.

## II. Design of Study

All the behavioural experiments were conducted in animal house laboratory in department of pharmacology in Indira Gandhi Medical College, shimla. The mice were reared in an initial environment for 6 weeks [6]. The age group selected for the study was 8 months, six male and six females were taken in the study, with weight ranging between 20 -29 grammes and with free access to food and water. After 6 weeks of rearing subjects were exposed to environmental change like social isolation in which each of the 12 mice were kept in separate cages hence one in each cage and they were housed alone with free access to sunlight, food and water. The subjects were housed in these conditions for 10 days and then behavioural experiments were performed to examine any changes in animal behaviours by changing the environments. After 10 days the animals were housed in other cages containing normally housed animals in a family. After one week of habituation in their new environment, we performed behavioural experiments to examine whether the animals behaviour were affected by these changes [7], [8].

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## III. Results

On the first day of experimentation we exposed a test group and control group to open field and observed their locomotive behaviour and observed that animals became more depressed after isolation as line crossing (P=0.01), central square entry (P<.001), central square duration = (P=0.007), rearing (P=0.03), Stretch attend (P<.001), grooming (P<.001) & urination = (P=0.001) were observed. i.e. Tables I (A) and (B).

TABLE I (A)
Open Field Test Observations: (Test Group) (Anxiety)

| Animal | Line crossing seconds | Centre square entry per 15 min | Centre square duration per visit seconds | Rearing | Stretch attend | Grooming per 15 minutes | Urination | Defecation |
|---|---|---|---|---|---|---|---|---|
| 1 | 5 | 23 | 2 | 70 | 32 | 75 | 6 | 5 |
| 2 | 8 | 46 | 4 | 74 | 24 | 55 | 7 | 8 |
| 3 | 2 | 74 | 7 | 95 | 45 | 40 | 4 | 6 |
| 4 | 6 | 52 | 1 | 65 | 30 | 90 | 3 | 7 |
| 5 | 3 | 38 | 2 | 60 | 25 | 80 | 2 | 6 |
| 6 | 9 | 07 | 60 | 15 | 30 | 100 | 2 | 8 |
| 7 | 36 | 18 | 5 | 35 | 24 | 40 | 1 | 11 |
| 8 | 10 | 15 | 3 | 39 | 28 | 36 | 1 | 5 |
| 9 | 3 | 22 | 4 | 46 | 22 | 55 | 2 | 6 |
| 10 | 3 | 16 | 2 | 45 | 20 | 38 | 1 | 3 |
| 11 | 3 | 17 | 2 | 47 | 24 | 40 | 1 | 2 |
| 12 | 2 | 15 | 7 | 61 | 21 | 48 | 1 | 10 |

TABLE I (B)
Open Field Test Observations: (Control Group) (Anxiety)

| Animal | Line crossing seconds | Centre square entry per 15 min | Centre square duration per visit seconds | Rearing | Stretch attend | Grooming per 15 minutes | Urination | Defecation |
|---|---|---|---|---|---|---|---|---|
| 1 | 3 | 60 | 2 | 40 | 5 | 20 | - | 10 |
| 2 | 2 | 70 | 2 | 42 | 10 | 25 | - | 11 |
| 3 | 3 | 55 | 2 | 35 | 6 | 30 | - | 10 |
| 4 | 5 | 70 | 2 | 40 | 11 | 20 | - | 8 |
| 5 | 4 | 65 | 2 | 41 | 15 | 35 | 1 | 10 |
| 6 | 2 | 70 | 2 | 40 | 10 | 20 | 1 | 7 |
| 7 | 2 | 60 | 2 | 40 | 7 | 20 | - | 6 |
| 8 | 3 | 64 | 2 | 38 | 9 | 30 | - | 10 |
| 9 | 3 | 60 | 2 | 37 | 10 | 35 | - | 10 |
| 10 | 2 | 65 | 2 | 36 | 15 | 33 | - | 3 |
| 11 | 1 | 70 | 2 | 40 | 10 | 30 | - | 7 |
| 12 | 2 | 65 | 2 | 39 | 5 | 25 | - | 6 |

TABLE II
Aggressive Behaviours (Observation Time 10 Minutes)

| Test group | | | | | Control groups | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Animal | Latency to first attack(sec) | Number of attacks | Duration of each fight (sec) | Number of body scars | Animal | Latency to first attack(sec) | Number of attacks | Duration of each fight (sec) | Number of body scars |
| 1 | 45 | 26 | 7 | 8 | 1 | 60 | 10 | 5 | 5 |
| 2 | 40 | 30 | 10 | 6 | 2 | 65 | 15 | 5 | 2 |
| 3 | 90 | 15 | 15 | 5 | 3 | 120 | 8 | 7 | 3 |
| 4 | 50 | 30 | 13 | 8 | 4 | 65 | 7 | 8 | 5 |
| 5 | 56 | 35 | 12 | 10 | 5 | 63 | 10 | 5 | 6 |
| 6 | 53 | 30 | 15 | 8 | 6 | 65 | 15 | 9 | 5 |
| 7 | 57 | 26 | 15 | 7 | 7 | 64 | 12 | 5 | 3 |
| 8 | 55 | 32 | 10 | 6 | 8 | 50 | 11 | 7 | 4 |
| 9 | 60 | 28 | 18 | 5 | 9 | 55 | 10 | 6 | 3 |
| 10 | 62 | 26 | 15 | 7 | 10 | 70 | 7 | 9 | 2 |
| 11 | 60 | 28 | 17 | 6 | 11 | 72 | 6 | 10 | 3 |
| 12 | 60 | 26 | 16 | 7 | 12 | 65 | 8 | 5 | 3 |

TABLE III
TEST FOR SOCIAL INTERACTIONS IN MICE (OBSERVATION TIME 10 MINUTES)

| Test group | | | | | | Control group | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Animal | No. of approaches | Crawling on each other | Nose to nose sniffing | Genital sniffing | Total time spent together | Animal | No of approaches | Crawling on each other | Nose to nose sniffing | Genital sniffing | Total time spent together |
| 1 | 13 | 10 | 05 | 2 | 6 | 1 | 17 | 13 | 10 | 09 | 8 |
| 2 | 10 | 07 | 03 | 1 | 5 | 2 | 16 | 15 | 10 | 08 | 8 |
| 3 | 06 | 03 | 01 | 1 | 5 | 3 | 15 | 12 | 15 | 10 | 7 |
| 4 | 07 | 05 | 02 | 1 | 3 | 4 | 15 | 15 | 10 | 06 | 9 |
| 5 | 12 | 10 | 05 | 2 | 7 | 5 | 15 | 10 | 13 | 06 | 6 |
| 6 | 09 | 06 | 03 | 1 | 6 | 6 | 12 | 12 | 11 | 05 | 8 |
| 7 | 06 | 03 | 01 | 0 | 6 | 7 | 14 | 12 | 10 | 10 | 7 |
| 8 | 07 | 03 | 01 | 1 | 6 | 8 | 15 | 12 | 10 | 10 | 6 |
| 9 | 10 | 07 | 04 | 1 | 3 | 9 | 15 | 13 | 10 | 07 | 6 |
| 10 | 07 | 03 | 01 | 0 | 6 | 10 | 12 | 14 | 14 | 06 | 8 |
| 11 | 08 | 05 | 02 | 1 | 4 | 11 | 15 | 11 | 12 | 07 | 9 |
| 12 | 10 | 08 | 06 | 2 | 6 | 12 | 14 | 15 | 11 | 07 | 8 |

TABLE IV
TAIL SUSPENSION TEST (DEPRESSION) (OBSERVATION TIME 6 MINUTES)

| Test group | | Control group | |
|---|---|---|---|
| Animal | Immobility time | Animal | Immobility time |
| 1 | 46 | 1 | 40 |
| 2 | 30 | 2 | 40 |
| 3 | 50 | 3 | 40 |
| 4 | 56 | 4 | 30 |
| 5 | 50 | 5 | 34 |
| 6 | 50 | 6 | 40 |
| 7 | 40 | 7 | 42 |
| 8 | 40 | 8 | 30 |
| 9 | 30 | 9 | 36 |
| 10 | 46 | 10 | 40 |
| 11 | 44 | 11 | 40 |
| 12 | 85 | 12 | 40 |

TABLE V
RESULTS OF OPEN FIELD TEST

| Parameter | Group | Line crossing | Centre square entry | Centre square duration | Rearing | Stretch Attend | Grooming | Urination | Defecation |
|---|---|---|---|---|---|---|---|---|---|
| Mean | Test | 4.67±2.3 | 29.67±18.8 | 5.83±4.0 | 54.25±21.5 | 27.08±6.6 | 62.75±16.9 | 2.67±2.0 | 7.00±1.2 |
| | Control | 2.67±1.0 | 64.50±4.9 | 2.00±0.0 | 39.00±2.0 | 9.42±3.3 | 26.92±5.9 | .17±0.3 | 8.17±2.4 |
| p- value | | 0.01* | <0.001* | 0.007* | 0.03* | <0.001* | <0.001* | 0.001* | 0.15 |

The very same day tests for aggressive behaviour were conducted in animals that were kept isolated for six weeks and fighting attitude of animals was observed and compared with the control group. The results of this test indicated that social isolation increases aggressive behaviour in animals i.e. latency to first attack was not found to be statistically different in the two groups. Number of attack were significant increased in the test group (P<.001), duration and number of attacks and scar mark were significantly high in the test group (P<.01) i.e Table VI.

TABLE VI
RESULTS FOR TEST FOR AGGRESSIVE BEHAVIOUR

| Parameter | Group | Latency to first attack | No of attcks | Duration of each fight | No of body scars |
|---|---|---|---|---|---|
| Mean±S.D. | Test | 57.33±12.2 | 27.67±4.8 | 13.58±3.2 | 6.92±1.4 |
| | Control | 67.83±17.4 | 9.92±2.9 | 6.75±1.8 | 3.67±1.3 |
| P value | | 0.10 | <0.001* | <0.001* | <0.001* |

Test for social interaction were conducted in isolated animals and different parameters were compared with the control group. It was observed that the isolated animals interacted less than other animals as number of approaches, crawling, nose sniffing and total time spent together were higher in control group (P<.001).

TABLE VII
RESULTS FOR TEST FOR SOCIAL INTERACTIONS

| Parameter | Group | No. of approaches | crawling | nose | Genital | Total time |
|---|---|---|---|---|---|---|
| Mean±S.D. | Test | 8.75±2.3 | 5.83±2.6 | 2.83±1.8 | 1.08±0.6 | 5.25±1.2 |
| | Control | 14.58±1.4 | 12.83±1.6 | 11.33±1.7 | .92±0.6 | 7.50±1.0 |
| P value | | <0.001* | <0.001* | <0.001* | 0.54 | <0.001* |

After the open field test we performed the tail suspension test next day and it was observed that the isolation depressed the animal. The animal became depressed in the test group (P=0.04).

TABLE VIII
RESULTS FOR TAIL SUSPENSION TEST

| Parameter | Group | Tail suspension |
|---|---|---|
| Mean±S.D. | Test | 47.25±14.2 |
| | Control | 37.67±4.1 |
| P value | | 0.04 |

## IV. DISCUSSION

In the present study we found that social isolation elevated the anxiety level of the mice and also increased depression in mice [9]-[11]. The findings of the present study are in agreement with results from [12]-[14]. In our conditions social isolation elevated anxiety levels and also depression like behaviour. Some researchers have changed the rearing conditions like they changed the size of the cage and no effect on depression was seen. This study also highlights that isolated animals behave more aggressively and they interact less socially. All these results pave a way for further investigating the animals for drug dependence and role of isolation in drug dependence.

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