Effect of Restraint Stress on The Growth Rates of Young Wistar Rats.

Dhungel S[a], Bhattacharya S[b], and Shrestha RN[c]

Abstract

Introduction Stress is the phenomenal; any stimulus which displaces the state of normal physiological function can cause stress and has its effect in various forms. Somatic growth and development are also affected by stress affecting growth releasing hormone.

Objectives This study was conducted to observe growth pattern in normal and stressed condition in growing rats so as to compare the effect of stress between the sexes and to conclude which sex is more affected.

Methods Experiments were conducted to investigate the effect of restraint stress applied at different times of the light-dark cycle on growth rates (nose-rump, head & tail length and body weight of rats). Wistar strain of Albino rats were restrained for 30 minutes, 12 hourly from 3rd to 12th week postnatal in an indigenously made restraining device, which consisted of a wooden platform (50 ´ 50 cm) to which a wire-mesh was attached. Body weight (BW), nose-rump length (NR-L) tail length (TL) and head length (HL) was recorded at weekly interval both in males and females of control and experimental groups. Recorded parameters were subjected to statistical analysis between the groups and sexes.

Result Restraint stress induced significant weight loss in both sexes but was greater in male. Result also showed greater effect on growth rates viz. NR-L, HL and TL in male as compared to female.

Conclusion Correlating experimental findings with similar growth patterns in human, it is not wrong to comment that females can tolerate stressful situation more easily than males.

Key words Wistar rats, Restraint stress, Growth rates, Body weight and body size.

Introduction

Any stimulus that displaces the state of normal physiological function can cause "stress". Selye [1] sated that "stress" is the state of the organism subjected to pressure to which its homeostatic mechanism cannot readily enable it to adapt. Later in 1960 Selye[2] described " stress" is the consequence of the rate of wear and tear in the biological system.

Ganong[3] defines " stress" as those stimuli which increases ACTH is an intricate chain which begins with the inputs at the higher centre, that triggers the hypothalamus. In response to stress full stimuli hypothalamus releases corticotrophin releasing hormone (CRH) to stimulate the pituitary, which in turn releases ACTH. Thus, adrenal cortex is stimulated for release of cortisol and androgen precursors. ACTH and cortisol are secreted in episodic manner in response to stress.

Restraint stress has been proposed as an animal model of depression and anorexia nervosa, as many investigators have shown that stress suppresses food intake and body weight gain in rats[3,4]. The stress-induced reduction in food intake has been demonstrated both as maintained decrease in 24-h food intake during and after repeated daily restraint stress[3,4,5] and as an acute response in the hours immediately after a single stress[4,5]. Weight loss, induced by repeated restraint, appears after the first stress session and the rat continue to lose more weight with each exposure to stress. However, once

Corresponding Author: Dr. Shaligram Dhungel, **E-mail:** docsd2002@yahoo.com [a]Department of Anatomy BPKIHS, Dharan; [b]Department of Anatomy BP Koirala Institute of Health Sciences Dharan, Nepal; [c]Institute of Medicine, Kathmandu.

stress has ended, restraint rats fail to return to the body weight of control animals[3,4,5].

Central mechanism involved in the stress-induced inhibition of food intake have not been fully elucidated, but certain peptides and neurotransmitters are thought to be involved in response. It is well established that monoamines[6] and CRH[4,7] influence feeding behavior and affects the body as a whole as well as individuals organs depending upon the type and intensity of stress[3,7,8] .

This experiment was conducted with aims and objectives in terms of

1. To observe normal growth pattern in growing rats in their postnatal life.
2. To compare the effect of the stress on the growth pattern.
3. To compare the effect of stress between sexes and to observe which sex is more affected.

Materials and Method

Ethical clearance was taken from the research committee of B.P. Koirala Institute of Health Sciences, Dharan, Nepal.

1. Animal

Wistar strain of pregnant rats were monitored at the interval of 8 hour to observe and note the date of delivery. The date of delivery was recorded as zero day of pups life. From day zero till day 21 the young ones remained with their mothers. On 21st day litters were separated from their mother and kept in separate cages. Groups of control and experimental of both sexes consisting of five rats in each group were formed randomly.

2. Environment

The rats were maintained in well-ventilated room. Temperature ranged between and 17^0 and 26^0 Celsius. Doors and windows were closed during morning, evening and night hour to prevent them from colds. They were kept in natural source of light which was 12:12 hour L:D cycle.

Size of the cages for housing the rats were 40 ´ 25 ´ 16 cm. Cages contained paddy husk, which were changed on every 2nd day.

3. Diet Regimen

The rats were maintained on standard rat pallet diet and foods, such as soaked Bengal gram, carrot,and bread on an average of 10 to 15gm/Rat . They were also maintained on small meat pieces twice a week. Food and drinking water were provided ads-libitum. Drinking water was acidified with hydrochloric acid to give a pH of 2.0 -2.5 this was achieved by adding 2 ml of hydrochloric acid to 3 liters of tap water. The purpose of adding acid to water is to prevent massive bacterial proliferation in the water bottle.

4. Stress Regimen

From 21st day postnatal onwards experimental groups of rats were subjected to restraint stress in an indigenously made restraining device, which consisted of a wooden platform (50x50cm) to which a wire-mesh was attached. From the morning 21st day (weaning day) to 84th day (end of 12 weeks) the rats were subjected to restraint for 30 minutes every 12 hourly. The food and water were withdrawn from both groups (control and experimental) during stress presentation. After stress presentation rats were returned to their respective cages but food and water were restored only after another 30 minutes.

5. Data Collection

a. Body weight : The body weight was recorded in a sensitive single pan balance.

b. Nose-rump length : The measurement was taken by stretching a nylon thread from shout to the anal opening and further the length was measured in centimeter scale.

c. Tail length : The measurement was taken by stretching a nylon thread from anal opening to the tip of the tail and thread length was further noted on a plastic centimeter scale.

d. Head length : The head length was measured from the snout to the posterior margin of skull.

All the recorded parameters in control and experimental groups of both sexes were tested statistically (Student t test).

Results

Results were made on the observations (by weight and measurement) of growths of normal and stressed rats on body weight (BW), nose-rump length (NR-L), tail length (TL) and head length (HL).

1. Growths of rats under normal condition

i. Growth in body weight

It was observed that at birth the average BW of male and female was 5.01 (± 0.33) and 4.90 (± 0.13) gm. respectively. In the both sexes, body weight seemed to be double at 1st week. Significant difference in the body weight was noted from 7th week onward till 12th week and BW of female was always found to be less than that of male (Table-1).

ii. Growth in nose-rump length

NR-L in male and female was found to be increased approximately 5 times at 12th week from day zero. NR-L was always noted less in female but significantly less was observed only at 4th, 9th, 11th and 12th week of postnatal age (Table-2).

iii. Growth in tail length

At birth TL of male rats was found to be 1.64 cm and in female 1.63cm further which increased to 6.55 and 6.40 cm respectively on weaning day (D21). Thereafter the growth in the TL of both sexes slowed down but continued at a steady rate up to D56. Later on the increase in the TL was not regular, instead it was in bouts. When it was compared to male, female TL was found to be always less but significant difference was observed from D 49 till D 84 (Table-2).

iv. Growth in head length

The HL of male and female rats at birth was found 1.76 ± 0.5 and 1.69 ± 0.6 cm respectively. In both sexes on weaning day, there was increase of 73% of the HL that of at birth. It was also observed that in both sexes from day 77 the HL was same and there was no further increase till the end of the study period. Although head size of female was found to be less but significant difference was not observed till 6th week of postnatal life, when compared to the male. Thereafter, significant difference was noted in head size (Table-2).

2. Growth of the rats under stressed condition

The rats were subjected to stress from weaning day. Comparison of the control and experimental between the groups and sexes of rats were made from 3rd to 12th week.

i. Growth in body weight

The effect of stress on the BW in both sexes was discernible within one week of stress presentation and continued up to the 12th week.

Table-3 showed more effect of stress in male when compared to female.

ii. Growth in nose-rump length

In male the growth pattern of the NR-L was found same in case of BW; which remarkably different in female (Table-4).

The effect of stress in male was pronounced from 4th week to 12th weeks. In female, effect of stress was there but mild and not consistent. This mild effect was pronounced on 2nd, 3rd, 4th & 6th week only.

iii. Growth in tail length.

Effect of stress on TL in male was noticeable within one week of stress presentation but in female it was noticeable only after three weeks and effect was found to be mild for next three weeks and thereafter no significant differences were recorded in the control as well as in experimental groups of female.

From the observations made in this study it was noted that males were more affected from stress (Table-5).

iv. Growth in head length

The effect of stress on growth of the head length in both sexes was notable after three weeks of stress presentation. Thereafter in males, stress caused marked significant retardation in the growth of the head size. However in females the effect of stress on head size was very less till 8th week post-natal and then onwards and till the end of the study period no signs of stressor effect recorded (Table-6).

Discussion and Conclusion

Hughes and Janner advocated that age of the rat is better recognized from day of copulation[6], it was found in this study that this may be dubious as laps of few hours often removes the sign of copulation[7]. As for the post-natal age this problem did not arise, because the pregnant rats were observed every eight hourly thereby the zero day of post-natal age could be accurately noted.

As the study period ranged from new born to adulthood, rat pups in their pre-weaning period of life restraint stress could not be subjected. Therefore stress regimen was introduced from the weaning day till 12th week post-natal.

In this study average birth weight (male & female) was 4.75 gm, which is closely similar to the past reports[8,9]. In the first week of post-natal life, it doubled and by weaning day it was 5 times of birth weight. Donaldson[10] found the weight of male rat on weaning day 23.90 gm. However, it was much lower than the Lane-petter's[9] figure on day 21 (45.00 gm), and also

Hain's[11] report i.e. 35.40 ± 0.77 gm. In the present study rapid gain in body weight was observed between 49th and 63rd day and thereafter a moderate weight gain was registered till 84th day. During this period body weight of rats of this series surpassed the weight reported by Donaldson[10] & Hain[11]. In post-weaning period the body weight gain was severely retarted due to restraint stress. Hunter and Clegg[12] also reported severe effect of hypoxic stress during postnatal life and stated that the birth weight depends on timing, severity and duration of stress. Surprisingly the BW found on the 77th day in the present study is same which was stated by Lane-petter's[9] on 70th day.

As for the (NR-L) of the male rats from birth onward, hitherto, the literatures available were of Donaldson[10] Hain[11] reported NR-L as 21.5 cm where as Donaldson's report[10] was considerably lesser than that of Hain[11] and the present study. Findings of Roberts and Black wood[13] are based on linear measurement of skull recorded on the radiograph. Although, the study is valuable one because it gives the rate of growth in a micrometer per day from 21 to 480 days, it shows much variability from manually measured length of head as in this study.

Like the weight, the TL also doubles during the first week of post-natal life. It increased four folds by day 21 and continued to increase in a retarding order upto day 84 of post-natal life. Hughes and Janner [6] reported, in black-hooded rat, the tail length reaches it's peak by 55 p.n.

The present work substantiating a recent work of Marti et al[14] showing that the chronic stressor effect on body weight, and subsequently affecting body length, tail length and head length on both sexes. Observation made on this study revealed that under normal conditions all parameter recorded are lesser in female when compared to male Thus it may be concluded that males are more susceptible to stressor effect than females.

Table 1: Body weight and Nose-rump length in growing rats under normal condition.
(Values expressed as mean ± SD)

	Body Weight (gm)		Nose-rump length (cm)	
Age (Days)	**Male**	**Female**	**Male**	**Female**
0	5.01 NS	4.90 (±0.13)	4.42 (±0.13)	4.26 NS (± 0.19)
7	11.18*	10.15 (±0.13)	6.40 (±0.07)	5.90** (±0.18)
14	20.66 NS	19.17 NS (±1.79)	7.98 (±0.19)	7.70 NS (±0.43)
21	25.89 NS (±1.31)	23.00 NS (±2.68)	9.32 (±0.29)	9.20 NS (±0.32)
28	38.89 (±5.24)	35.00 NS (±2.82)	10.84 (±0.35)	10.50* (±0.54)
35	52.90 (±1.68)	47.50* (±4.25)	11.83(±0.40)	11.60 NS (±0.80)
42	80.00 (±1.43)	70.83** (±5.21)	12.80 (±0.21)	12.70 NS (± 0.33)
49	105.00 (± 1.41)	88.33*** (±6.05)	16.70 (±0.58)	16.06 NS (± 0.79)
56	140.00 (±2.16)	112.50*** (±8.21)	17.50 (±0.14)	16.88 NS (± 0.64)
63	180.00 (±3.43)	134.16***(±10.68)	19.00 (±0.30)	17.98** (±0.45)
70	200.00 (±4.40)	150.00***(±10.95)	19.00 (± 0.41)	18.16 NS (±0.66)
77	213.00 (±3.80)	155.00***(±13.41)	21.20 (± 0.22)	18.24*** (±0.27)
84	250.00(±7.90)	165.00***(±13.69)	21.40(±0.21)	19.38(±1.20)

NS : Not significant; * O< 0.05; ** P < 0.01; *** P < 0.001

Table 2: Tail and Head length in growing rat under normal condition.

(Values expressed as mean ± SD)

	Tail Length (cm)		Head Length (cm)	
Age				
(Days)	**Male**	**Female**	**Male**	**Female**
0	1.64 (± 0.11)	1.63 NS (±0.10)	1.76 (±0.05)	1.69NS (±0.06)
7	3.02 (±0.10)	2.97 NS (±0.9)	2.50 (±0.07)	2.34 NS (±0.11)
14	5.42 (±0.16)	5.30 NS (±0.21)	3.06 (± 0.05)	3.02 NS (±0.04)
21	6.55 (± 0.15)	6.40 NS (±0.16)	3.26(±0.19)	3.20 NS (±0.14)s
28	8.56 (±0.23)	8.40 NS (±0.37)	3.59 (±0.05)	3.50 NS (±0.07)
35	9.33 (±0.21)	9.00 NS (±0.27)	3.78 (±0.31)	3.7 NS (±0.24)
42	12.00 (±35)	11.70 NS (±0.35)	4.10(± 0.14)	4.01 NS (±0.08)
49	14.20 (±0.21)	12.81**(±0.67)	4.50 (±0.14)	4.26*(±0.08)
56	14.70 (± 0.26)	13.86** (±0.42)	4.70 (±0.14)	4.41** (±0.04)
63	16.00 (± 0. 46)	14.45**(±0.56)	4.80 (±0.14)	4.41** (±0.07)
70	16.30 (±0.50)	15.31* (±0.75)	5.00 (±0.14)	4.54***(±0.12)
77	17.40 (±0.39)	15.50*** (±0.69)	5.10 (±0.23)	4.69* (±0.16)
84	18.00 (± 0.25)	15.56***(±0.62)	5.10 (±0.15)	4.69* (±0.16)

NS - Not significant; * P < 0.05; ** P < 0.01 *** P < 0.001

Table 3: Effect of restraint stress on the body weight of rats.

(Values are expressed as mean ±SD in gm.)

	Male		Female	
Age				
(days)	**Control**	**Restraint**	**Control**	**Restraint**
28	38.89±5.24	27.24*±1.91	35.00±2.82	30.10*±1.74
35	52.90±1.68	37.70***±2.25	47.50±4.25	42.44*±1.37
42	80.00±1.43	46.90***±2.19	70.83±5.21	53.04***±1.79
49	105.00±1.41	72.94***±4.17	88.33±6.05	76.80**±2.09
56	140.00±2.16	97.46***±5.56	112.50±8.21	98.26**±1.01
63	180.00±3.43	114.78***±2.22	134.16±10.95	108.44***±1.40
70	200.00±4.40	128.00***±4.12	150.00±10.95	120.00***±2.54
77	213.00± 3.80	135.00**±3.80	155.00±13.41	124.00***±2.88
84	250.00±7.90	149.40***±3.78	165.00±13.69	130.00***±5.00

NS; Not significant; * P < 0.05; **P <0.01; *** P < 0.001

Table 4: Effect of restraint stress on the nose-rump length of rats.

(Values are expressed as Mean ±SD in cm.)

	Male		Female	
Age (days)	**Control**	**Restraint**	**Control**	**Restraint**
28	10.84±0.35	9.20*±0.29	10.50±0.54	10.00 NS±0.41
35	11.83±0.40	10.76***±0.19	11.60±0.80	10.20*±0.21
42	12.80±0.21	11.30**±0.29	12.70±0.33	11.40*±0.29
49	16.70±0.58	14.20***±0.37	16.06±0.79	14.00**±0.5
56.	17.50±0.14	15.66***±0.08	16.88±0.64	16.70 NS±0.10
63	19.00±0.30	17.06***±0.24	17.98±0.45	16.96*±0.39
70	19.00±0.81	18.10*±0.47	18.16±0.66	17.90 NS±0.25
77.	21.20±0.22	19.10***±0.52	18.24±0.27	18.10 NS±0.67
84	21.40±0.21	19.50***±0.21	19.38±1.20	18.54 NS±0.37

NS: Not significant; * P < 0.05; ** P< 0.01; *** P < 0.001

Table 5: Effect of restraint stress on the tail length of rats.

(Values are expressed as Mean ±SD in cm.)

	Male		Female	
Age (days)	**Control**	**Restraint**	**Control**	**Restraint**
28	8.56±0.33	8.00**±0.20	8.40±0.37	8.20NS±0.22
35	9.13±0.41	8.70**±0.20	9.00±0.27	8.50NS±0.15
42	12.00±0.35	9.82***±0.25	11.70±0.35	10.00**±0.51
49	14.20±0.21	11.32***±0.46	12.81±0.67	11.60*±0.29
56	14.70±0.26	13.10***±0.15	13.86±0.42	12.70*±0.15
63	16.00±0.46	13.72***±0.27	14.15±0.46	13.80 NS±0.29
70	16.30±0.50	14.86**±0.45	15.31±0.75	14.50 NS±0.25
77	17.40±0.38	15.40**±0.23	15.50±0.69	14.80 NS±0.16
84	18.00±0.25	15.78***±0.19	15.56±0.62	15.50 NS±0.19

NS: Not Significant; * P < 0.05; **P < 0.01; ***P < 0.001 ***P < 0.001

Table 6: Effect of restraint stress on the head length of rats

(Values are expressed as Mean ±SD in cm.)

	Male		Female	
Age				
Days	**Control**	**Restraint**	**Control**	**Restraint**
28	3.59±0.05	3.48*±0.05	3.50±0.07	3.45*±0.06
35.	3.78±0.31	3.72 NS ±0.08	3.70±0.24	3.70 NS ±0.07
42	4.10±0.14	3.80**±0.07	4.01±0.08	3.89*±0.10
49	4.50±0.14	4.10***±0.07	4.26±0.08	4.99*±0.07
56	4.70±0.14	4.22***±0.08	4.41±0.04	4.20*±0.15
63	4.80±0.14	4.46**±0.11	4.41±0.07	4.38 NS ±0.13
70	5.00±0.14	4.50***±0.07	4.54±0.12	4.40 NS ±0.08
77	5.10±0.23	4.54**±0.11	4.60±0.08	4.46 NS ±0.06
84	5.10±0.15	4.70***±0.07	4.76±0.16	4.62 NS ±0.08

NS: Not significant; * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$

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References

1. Selye H. The general adaption syndrome and disease of adaption. *J Clin endocrine Metab* 1946; 6:117.
2. Seley H. The concept of stress in experimental physiology. In: Stress and Psychiatric Disorder (Ist edn; ch.II;). Blackwell Scientific Publication, Oxford 1960:67-75.
3. Ganong W.F.: Review of Medical Physiology (17th edn). Prentice-Hall Internationl Inc. 1995: 327-51.
4. Kennett G.A, Chauloff F., Marcou M., and Curzon G.: Female rats are more vulnerable than males in an animal model of depression: the possible role of serotinin. Brain Res. 1986; 382: 416-21.
5. Kratin D.D, Gosnell B.A. and Majchrzak M.J.: The anorexic effect of CRH and restraint stress decrease with repeated exposures. Biol. Physchiatry. 1990;27:1094-1102.
6. Hughes P.C.R. and Janner J.M.: A longitudinal study of the growth of the black-hooded rat: Methods of measurement and rate of growth for skull, limb, pelvis, nose-rump and tail length. *J. Anat.* 1970; 106:349-70.
7. Bennett J.P. and Vickery, B.H.: rats and Mice. In: Reproduction and breeding techniques for laboratory animals; Editor Hafer E.S.E., Lea and Febiger. Philadelphia, 1970; 299-315.
8. William S.S.: Hand book of biological data. WB Saunders Co. Philadelphia, London 1956:174.
9. Lane-Petter W.: The Laboratory Rat. In: The UFAW Hand book on the care and management of laboratory animal (5th edn.) London: Churchill Livingstone 1976:204-11.
10. Donaldson H.H.: The rat. Quoted by Hain A.M. Anat Rec 1934;59:383-91.
11. Hain A.M.: Some facts regarding growth of wistar rat under standard conditions in Britain. *Anat Rec* 1934; 59:383-91.
12. Hunter C. and Clegg E.J.: Changes in body weight of the growing and adult mouse in response to hypoxic stress. *J Anat* 1973; 114:185-99.
13. Roberts G.J. and Black-Wood J.J.: Growth of the cartilages of midline base: a radiographic and histological study. *J Anat* 1983; 136: 307-20.
14. Marti O., Marti J. and Armario A.: Effect of chronic stress on food int ake in rats. Influence of stressor intensity and duration of daily exposure. *Physiol Behav* 1994;55 (II): 747-54.