INFLUENCE OF ISOLATION STRESS ON MONOAMINE LEVELS IN DISCRETE REGIONS OF THE RAT BRAIN CORRELATED WITH BEHAVIOUR

N. SUTHANTHIRARAJAN AND SARADA SUBRAHMANYAM

Department of Physiology,
Post-Graduate Institute of Basic Medical Sciences,
University of Madras, Taramani – Madras – 600 113

(Received on May 4, 1982)

Summary : Various forms of stressful stimuli have been shown to affect brain catecholamine (CA) and indoleamine (IA) levels, although the literature contains some conflicting views Changes in monoamine levels were also found to accompany the behavioural changes in animals. In the present study an attempt was made to correlate the behavioural changes with the monoamine level in discrete regions of rat brain after different periods of isolation stress. The results clearly indicate that not only the central norepinephrine and dopamine neurons involved in the expression of aggressive behaviour after isolation stress but also the serotoninergic neurons might take part.

Key words : isolation stress, monoamines, discrete regions, behavioural changes, rat brain

INTRODUCTION

Prolonged social isolation is a well known method to induce aggressiveness and other behavioural abnormalities in male animals of various species. This phenomenon has been studied in mice (28, 29, 35), monkeys (15) and in rats (16). With prolonged isolation Geller *et al.* (11) found an increase in the brain nor-epinephrine (NE) of rats. In mice, Welch and Welch (33, 34) found a decrease in turnover rate of both NE and serotonin. Modigh (21) found a decrease in the synthesis rate of catecholamines. Because of the above suggested interrelationship between biogenic amines and emotional behaviour observed following social isolation, we studied the levels of nor-epinephrine, dopamine, epinephrine, serotonin and its metabolite 5-HIAA in discrete regions of rat brain in different periods of isolation stress. In the present study the monoamine response is correlated with the behaviour pattern exhibited by the individual animal.

MATERIAL AND METHODS

Early adolescent male albino rats of Wistar strain weighing 100-120 *gm* were chosen and divided into two groups. The animals were fed with standard food pellets (Hindustan Lever Ltd., India) and water was made available to them *ad libitum.*

Group-I (Control): This group consists of twelve rats each and they were not subjected to any kind of stressful situations. The animals were housed six in each cage (40 x 30 x 25 cms.). They were sacrificed by decapitation at the end of 6, 13 and 26 weeks respectively.

Group - II : Twentyseven male rats were divided into three sub-groups of nine each. Rats from this sub-group were kept individually in metallic isolation cage (40x30x25 *cms*) which faced a blank white wall so that, the animal did not have any visual contact with each other. They were exposed to isolation stress for a period of 6, 13 and 26 weeks. At the end of the specific periods of isolation, the rats were decapitated and their brains were removed. The discrete regions of the brain were dissected out according to the method of Glowinski *et al.* (13) and weighed in a mettler balance. The concentration of nor-epinephrine, epinephrine, dopamine, serotonin and its metabolite 5-hydroxyindole acetic acid was assayed by spectrofluorimetric method (17). The plasma corticosterone was estimated by the method of Mattingly (19). The spontaneous motor activity of single isolated rat was measured after 6 and 26 weeks, using photoactometer supplied by Techno Electronics, Lucknow.

RESULTS

Table I shows the mean values of NE, E and DA concentration in grouped and isolated rats in various dissected areas. The levels of NE showed a significant ($P<0.01$) decrease at the end of 6 and 13 weeks of isolation in hypothalamus (HPT), pons-medulla (PM) and a significant ($P<0.01$) increase at the end of 26 weeks of isolation in all the regions except cerebral cortex (CC). However, in midbrain the increase was seen even from 6th week of isolation.

Epinephrine level revealed a gradual rise at the end of 6 and 13 weeks followed by a significant ($P<0.01$) increase at the end of 26 weeks of isolation in striatum, hypothalamus, pons-medulla, cerebral cortex and cerebellum.

Dopamine level showed a reduction in 6 and 13 weeks of isolation. The reduced level persisted even after chronic isolation of 26 weeks in all the regions except midbrain where the level showed a rise in all the three periods.

TABLE I : Effect of different periods of isolation stress on the concentration of nor-epinephrine (NE), epinephrine (E), dopamine (DA) levels ($\mu g/g$ wet tissue) in discrete regions of the rat brain compared with control (mean $\pm$ S.E.M.)

Areas	*Isolation stress in weeks*	*Nor-epinephrine*	*Epinephrine*	*Dopamine*
Striatum (STM)	0 (15)	0.578±0.102	0.282±0.051	2.993±0.517
	6 (9)	0.451±0.095	0.302±0.055	2.075±0.395
	13 (9)	0.721±0.131	0.311±0.060	1.043±0.195*
	26 (9)	1.801±0.343*	0.534±0.107*	1.743±0.319*
Hypothalamus (HPT)	0	1.127±0.250	0.317±0.062	2.835±0.495
	6	0.341±0.094*	0.333±0.070	2.079±0.397
	13	0.453±0.100*	0.398±0.089	1.318±0.201*
	26	2.144±0.316*	0.613±0.137*	0.971±0.123*
Midbrain (MB)	0	0.468±0.085	0.197±0.029	0.671±0.117
	6	0.755±0.143*	0.210±0.031	2.553±0.601**
	13	1.033±0.376**	0.227±0.030	3.147±0.703**
	26	0.943±0.295*	0.251±0.034	2.043±0.394
Pons-medulla (PM)	0	0.456±0.067	0.123±0.028	2.061±0.357
	6	0.251±0.074*	0.201±0.033	1.695±0.198
	13	0.241±0.084*	0.269±0.047	0.713±0.131**
	26	0.668±0.089*	0.294±0.067*	0.304±0.044**
Cerebral Cortex (CC)	0	0.210±0.051	0.052±0.019	
	6	0.136±0.025	0.144±0.054*	
	13	0.145±0.029	0.139±0.048*	—
	26	0.257±0.054	0.176±0.032*	
Cerebellum (C)	0	0.265±0.071	0.175±0.065	
	6	0.192±0.044	0.301±0.081*	
	13	0.251±0.069	0.340±0.079*	—
	26	1.072±0.234**	0.434±0.107*	

Number in parentheses refer to number of animals

*$P<0.01$; **$P<0.001$.

TABLE II : The concentration of 5–hydroxytryptamine (5–HT) and 5–hydroxyindole acetic acid (5–HIAA) levels ($\mu g/g$) after 6, 13 and 26 weeks of isolation stress in discrete regions of the rat brain (mean $\pm$ S.E.M.).

Areas	*Isolation stress in weeks*	*5–HT*	*5–HIAA*
Striatum (STM)	0 (15)	0.402 ± 0.082	0.641 ± 0.103
	6 (9)	$0.122 \pm 0.038^{*}$	0.517 ± 0.100
	13 (9)	$0.129 \pm 0.039^{*}$	0.496 ± 0.101
	26 (9)	0.416 ± 0.104	$2.601 \pm 0.453^{**}$
Hypothalamus (HPT)	0	0.515 ± 0.115	1.054 ± 0.217
	6	$0.123 \pm 0.075^{**}$	0.759 ± 0.122
	13	$0.217 \pm 0.088^{*}$	0.673 ± 0.112
	26	$0.793 \pm 0.142^{*}$	$5.294 \pm 0.853^{**}$
Midbrain (MB)	0	0.346 ± 0.073	1.131 ± 0.341
	6	$0.101 \pm 0.031^{*}$	1.237 ± 0.376
	13	$0.127 \pm 0.037^{*}$	1.329 ± 0.417
	26	$0.547 \pm 0.121^{*}$	$5.073 \pm 0.819^{**}$
Pons–medulla (PM)	0	0.362 ± 0.081	0.985 ± 0.173
	6	$0.104 \pm 0.041^{*}$	1.123 ± 0.182
	13	$0.072 \pm 0.035^{*}$	1.540 ± 0.208
	26	$0.578 \pm 0.105^{*}$	$4.530 \pm 0.769^{**}$
Cerebral Cortex	0	0.074 ± 0.019	0.744 ± 0.139
	6	0.069 ± 0.017	0.593 ± 0.111
	13	0.052 ± 0.017	0.555 ± 0.105
	26	$0.176 \pm 0.043^{*}$	$1.734 \pm 0.225^{*}$
Cerebellum (C)	0	0.103 ± 0.026	0.474 ± 0.094
	6	0.095 ± 0.020	0.481 ± 0.101
	13	0.155 ± 0.061	0.399 ± 0.087
	26	0.131 ± 0.037	$0.834 \pm 0.121^{*}$

Number in parentheses refer to number of animals.

$^{*}P < 0.01$; $^{**}P < 0.001$

5-HT and 5-HIAA levels are summarized in Table II. The concentration of 5-HT showed a decrease at the end of 6 and 13 weeks of isolation but a marked increase at the end of 26 weeks of isolation especially in hypothalamus, midbrain and pons-medulla.

Dopamine level showed a reduction in 6 and 13 weeks of isolation. The reduced level persisted even after chronic isolation of 26 weeks in all the regions except midbrain where the level showed a rise in all the three periods.

TABLE I : Effect of different periods of isolation stress on the concentration of nor-epinephrine (NE), epinephrine (E), dopamine (DA) levels ($\mu g/g$ wet tissue) in discrete regions of the rat brain compared with control (mean $\pm$ S.E.M.)

Areas	*Isolation stress in weeks*	*Nor-epinephrine*	*Epinephrine*	*Dopamine*
Striatum (STM)	0 (15)	0.578±0.102	0.282±0.051	2.993±0.517
	6 (9)	0.451±0.095	0.302±0.055	2.075±0.395
	13 (9)	0.721±0.131	0.311±0.060	1.043±0.195*
	26 (9)	1.801±0.343*	0.534±0.107*	1.743±0.319*
Hypothalamus (HPT)	0	1.127±0.250	0.317±0.062	2.835±0.495
	6	0.341±0.094*	0.333±0.070	2.079±0.397
	13	0.453±0.100*	0.398±0.089	1.318±0.201*
	26	2.144±0.316*	0.613±0.137*	0.971±0.123*
Midbrain (MB)	0	0.468±0.085	0.197±0.029	0.671±0.117
	6	0.755±0.143*	0.210±0.031	2.553±0.601**
	13	1.033±0.376**	0.227±0.030	3.147±0.703**
	26	0.943±0.295*	0.251±0.034	2.043±0.394
Pons-medulla (PM)	0	0.456±0.067	0.123±0.028	2.061±0.357
	6	0.251±0.074*	0.201±0.033	1.695±0.198
	13	0.241±0.084*	0.269±0.047	0.713±0.131**
	26	0.668±0.089*	0.294±0.067*	0.304±0.044**
Cerebral Cortex (CC)	0	0.210±0.051	0.052±0.019	
	6	0.136±0.025	0.144±0.054*	
	13	0.145±0.029	0.139±0.048*	—
	26	0.257±0.054	0.176±0.032*	
Cerebellum (C)	0	0.265±0.071	0.175±0.065	
	6	0.192±0.044	0.301±0.081*	
	13	0.251±0.069	0.340±0.079*	—
	26	1.072±0.234**	0.434±0.107*	

Number in parentheses refer to number of animals

*$P<0.01$; **$P<0.001$.

April–June 1983
Ind. J. Physiol. Pharmac.

TABLE II : The concentration of 5–hydroxytryptamine (5–HT) and 5–hydroxyindole acetic acid (5–HIAA) levels ($\mu g/g$) after 6, 13 and 26 weeks of isolation stress in discrete regions of the rat brain (mean $\pm$ S.E.M.).

Areas	*Isolation stress in weeks*	*5–HT*	*5–HIAA*
Striatum (STM)	0 (15)	0.402±0.082	0.641±0.103
	6 (9)	0.122±0.038*	0.517±0.100
	13 (9)	0.129±0.039*	0.496±0.101
	26 (9)	0.416±0.104	2.601±0.453**
Hypothalamus (HPT)	0	0.515±0.115	1.054±0.217
	6	0.123±0.075**	0.759±0.122
	13	0.217±0.088*	0.673±0.112
	26	0.793±0.142*	5.294±0.853**
Midbrain (MB)	0	0.346±0.073	1.131±0.341
	6	0.101±0.031*	1.237±0.376
	13	0.127±0.037*	1.329±0.417
	26	0.547±0.121*	5.073±0.819**
Pons–medulla (PM)	0	0.362±0.081	0.985±0.173
	6	0.104±0.041*	1.123±0.182
	13	0.072±0.035*	1.540±0.208
	26	0.578±0.105*	4.530±0.769**
Cerebral Cortex	0	0.074±0.019	0.744±0.139
	6	0.069±0.017	0.593±0.111
	13	0.052±0.017	0.555±0.105
	26	0.176±0.043*	1.734±0.225*
Cerebellum (C)	0	0.103±0.026	0.474±0.094
	6	0.095±0.020	0.481±0.101
	13	0.155±0.061	0.399±0.087
	26	0.131±0.037	0.834±0.121*

Number in parentheses refer to number of animals.

*$P<0.01$; **$P<0.001$

5-HT and 5-HIAA levels are summarized in Table II. The concentration of 5-HT showed a decrease at the end of 6 and 13 weeks of isolation but a marked increase at the end of 26 weeks of isolation especially in hypothalamus, midbrain and pons-medulla.

The level of 5-HIAA did not reveal any change in acute stages whereas, at the end of 26 weeks, the levels showed a significant ($P<0.001$) rise above those of the controls.

Effect of isolation stress on total wet weight of the brain (added the discrete regions) is shown in Fig. 1. The weight showed a rise at the end of 6 and 13 weeks of isolation. The plasma corticosterone level after isolation stress showed a rise and remained elevated at the end of 26 weeks of isolation.

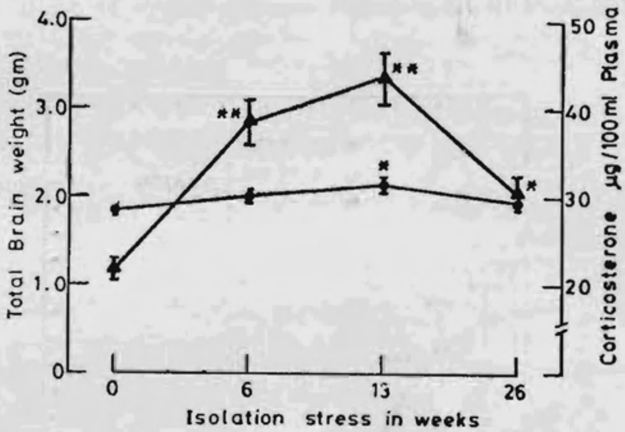

Fig. 1 : Effect of isolation stress on total brain weight (gm) (●—●) and plasma corticosterone level (µg%) of rat compared with control.
Vertical bar indicates SEM
*$P<0.01$; **$P<0.001$

Behavioural observation : The spontaneous motor activity was higher in isolated than in grouped rats (Fig. 2). The animals in this group became very much excited, aggressive and hyperactive. The interesting observation especially in the acute isolated group (6 weeks) was that 70% of the animals showed the habit of keeping the food pellets in one corner of the cage during night.

DISCUSSION

Brain catecholamines are altered by stress, resulting in decreased NE concentration (2, 4, 23) and increased NE turnover (6, 14, 18, 27). Dopamine concentrations after stress have been reported as unchanged (2, 4, 6, 12, 14) while DA turnover was increased (2), decreased (6, 18) or unchanged (14, 27). In the present study the decreased level of NE and DA in acute stress may be due to the enhanced release by the increased neuronal activity (14). It is also possible that the acute streess in these regions could result in fatigued synthetic mechanism whereby release and synthesis of the transmitter do not go hand-in-hand. In chronic isolation the level of NE and E showed a rise probably due to increased resynthesis rather than decreased metabolism to maintain the interneurotransmitter balance. The decreased level of DA persisted even after 26 weeks of isolation stress suggested that the resynthesis mechanism (more conversion of DA into NE and E) might have been triggered to meet the emergency demand. The only region which showed a rise in the level of NE and DA was midbrain, the cause for which is yet to be established.

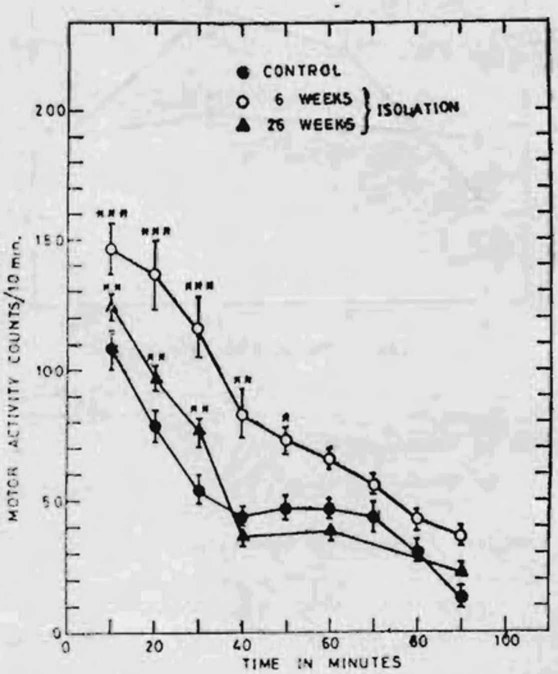

Fig. 2 : Spontaneous motor activity in single isolated rat after 6 and 26 weeks compared with control each point represents mean value of 6 determinations.

$^{*}P<0.05$, $^{**}P<0.01$, $^{***}P<0.001$

Vertical represents SEM.

After stress, concentration of 5-HT have been reported as unchanged (2), increased (26) or decreased (5, 7, 30). 5-HT metabolism was observed to be accelerated in the CNS following various stressors (1,2,3,8,26,32). In the present study the level of 5-HT declined significantly from the control suggesting the endogenous release of 5-HT from the terminals. The level after chronic exposure showed a rise probably due to resynthesis mechanism. The level of 5-HIAA did not reveal any change in the acute conditions as reported by Maynert *et al.* (20. Whereas, it was elevated after chronic isolation parallel to 5-HT level. This suggests the simultaneous activation of both synthesis and metabolism of erotoninergic neurons after chronic isolation stress.

Although the brains looked similar, yet acute isolation stress caused an increase in the total wet weight of the brain (added the dissected regions). It appears from the results that acute isolation can alter the brain weight which inturn might influence the brain function.

Isolation stress caused activation of pituitary adrenocortical-axis by increased plasma corticosterone level. There are evidences that cortisol may play a role in the restoration of brain serotonin levels (24, 25, 31) and this may mediate the negative feed-back action of glucocorticoids. This is supported by the findings that steroid sensitive neurons are present in different parts of the brain (22).

In our studies the spontaneous motor activity was higher in acute isolated than the chronic isolated group of rats. This may be responsible for the hyperactivity and aggressive behaviour exhibited by this group. Similar observations have been made in mice (9, 10). All the above evidence point out that not only the central NE and DA neurons that may be involved in the expression of aggressive behaviour after isolation stress but also the serotoninergic neurons might take part.

REFERENCES

1. Bliss, E.L. Effect of behavioral manipulations upon brain serotonin and dopamine In : Barchas, J. and E. Usdin (Eds.) Serotonin and Behaviour. Academic Press, New York, 1973.
2. Bliss, E.L., J. Aillion and J. Zwanziger. Metabolism of norepinephrine, serotonin and dopamine in rat brain with stress. *J. Pharmac. Exp. Ther.*, **164** : 122–134, 1968.
3 Bourgoin, S., Y. Morot-Gaudry, J. Glowinski and M. Hamon. Stimulating effect of short term ether anaesthesia on central 5-HT synthesis and utilization in mouse brain. *Europ. J. Pharmac*, **22** : 209, 1973.
4. Carr, L.A. and K.E. Mocre. Effects of reserpine and α–methylthyrosine on brain catecholamines and the pituitary-adrenal response to stress. *Neuroendocrinology*, **3** : 285–302, 1969.
5. Corrodi, H., K. Fuxe and T. Hokfelt The effect of immobilization stress on the activity of central monoamine neurons. *Life Sci.*, Part I, **7** : 107, 1968
6. Corrodi, H., F. Fuxe, P. Lidbrink and L. Olson. Minor tranqulizers, stress and central catecholamine neurons. *Brain Res.*, **9** : 1–16, 1971.
7. Curzon, G. and A.R. Green. The effect of immobilization on rat liver tryptophan hydroxylase and brain 5-hydroxytryptamine metabolism. *Brit. J. Pharmac.*, **37** : 689, 1969.
8. Curzon, G., M.H. Joseph and P.J. Knott. Effect of immobilization and food deprivation on rat brain tryptophan metabolism. *J. Neurochem.*, **19** : 1967, 1972.
9. Essman, W.B. The development of activity in isolated and aggregated mice. *Anim. Behav.*, **14** : 406–409, 1966.
10. Essman, W.B. Difference in locomotor activity and brain serotonin metabolism in differentially housed mice. *J. Comp. Physiol. Psychol.* **66** : 244–246, 1968.
11. Geller, E., A. Yuwiller, and J.F. Zolran. Effects of environmental complexity on constituents of brain and liver. *J. Neurochem.*, **12** : 949–953, 1965.

13. Glowinski, J. and L. Iversen Regional studies of catecholamines in the rat brain. I. The disposition of (3H) norepinephrine (3H) dopamine and (3H) dopa in various regions df the brain. *J. Neurochem.*, **13** : 655–669, 1966.

14. Cordon, R., S. Spector, A. Sjoerdsma and S. Udenfriend, Increased synthesis of norepinephrine and epinephrine in the intact rat during exercise and expcsure to cold. *J. Pharmac. Exp. Ther.*, **153** : 440–447, 1966.

15. Harlow, H.F., R.O. Dosworth and M.K. Harlow. Total social isolation in monkeys. *Proc. Nat. Acad. of Sci.*, **54** : 90–97, 1965.

16. Johnson, R.N., M.J. DeSisto, and A. Koenig. Social experience and intraspecies aggression in rats. *Proc. 78th Ann. Conv. of Amer. Psychol. Acssoc.*, **5** : 231–232, 1970.

17. Kari, H.P., P.P. Davidson, H.H. Kohland and M.H. Kcchhar. Effects of ketamine on brain monoamine levels in rats. *Res. Comm. in Chemical. Path. and Pharmacology*, **20** (3) : 475–488, 1978.

18. Lidbrink, P., H. Corrodi, K. Fuxe and L. Olson. Barbiturates and meprobamate : decrease in catecholamine turnover of central doapine and noradrenaline neuronal systems and the influrnce of immobilizaticn stress. *Brain Res.*, **45** : 507–524, 1972.

19. Mattingly, D. A simple fluorimetric methcd for the study of free 17-hydroxy corticosterοids in human plasma. *J. Clin, Pathol.*, **15** : 374, 1962.

20. Maynert, E.W., R. Levi. Stress induced release of brain norepinephrine and its inhibition of drugs. *J. Pharmac. Exp. Ther.*, **143** : 90–95, 1964.

21. Modigh, K. Effects of isolation and fighting in mice on the rate of synthesis of noradrenaline, dopamine and 5-hydroxytryptamine in the brain. *Psychopharmacologia (Bcrl.)* **33** : 1–17, 1973.

22. Steiner, F.A., K. Ruf and K. Akert. Steroid sensitive neurons in rat brain anatomical localization and responses to neurohumors and ACTH. *Brain Res.*, **12** : 74–85, 1969.

23. Stone, E.A. Accumulation and metabolism norepinephrine in rat hypothalamus after exhaustive stress. *J. Neurochem.*, **21** : 589–601, 1973.

24. Telegdy, G., I. Vermes, and K. Lissak. Changes induced by stress and corticosteroid treatment in the activity of the brain serotoninergic system. *XXVIth Int. Cong. Phys. Sci., New Delhi,* Vol. XI pp. 292, 1974.

25. Telegdy, G. and I. Vermes. Changes induced by stress in the activity of the serotonergic system in limbic-brain structures. In : Catecholamine and stress ed. by Usdin, Kvetnansky and Kopin (N.Y.) pp. 145, 1975.

26. Thierry, A.M., M. Fekete and J. Glowinski. Effects of stress on the metabolism of noradrenaline, dopamine and serotonin in the central nervous system of rat. II. Modification of serotonin metabolism. *Europ. J. Pharmac.*, **4** : 385, 1968.

27. Thierry, A.M., F. Javoy, J. Glowinski and S.S. Kety. Effects of stress on the metabolism of norepinephrine, dopamine and serotonin in the central nervous system of the rat. I. Modifications of norepinephrine turnover. *J. Pharmac. Exp. Ther.*, **163** : 163–171, 1968.

28. Valzelli, L. Drugs and aggressiveness. *Advan. Pharmacol.*, **5** : 79–108, 1967.

29. Valzelli, L. Aggressive behaviour induced by isolation. In : Proc. Int. Symp. on the Biology of Aggressive Behaviour, pp. 70–76 (Eds) S. Garattini and E.B. Sigg., *Excerpta Medica Foundation, Amsterdam,* 1969.

30. Vermes, I., G. Telegdy and K. Lissak, Correlation between hypothalamic serotonin content and adrenal function during acute stress. Effect of adrenal corticosteroids on hypothalamic serotonin content. *Acta Physiol. Acad. Sci. Hung.*, **43** : 33, 1933.

31. Vermes, I. and G. Telegdy. The role of corticcsteroids in the stress-induced alteration of the serotonin and 5-hydroxyindole acetic acid content in the limbic system in rats. *J. Steroid Biochem.*, **5** : 397, 1974.

32. Weiss, B.L. and G.K. Aghajanian. Activation of brain serotonin metabolism by heat : Role of midbrain raphe neurons. *Brain Res.* **26** : 37–42, 1971.

33. Welch, B.L. and A.S. Welch. A greater lowering of brain and adrenal catecholamines in group housed than in individually–housed mice administered DL–α-methyl-tyrosine. *J. Pharm. Pharmac.* **20** : 244–246 1968.

34. Welch, B.L. and A.S. Weleh. Aggression and the biogenic amine neurohumors. In : Proc. Int. Sympo. on the Biology of Aggressive Behaviour, pp. 188–202, (Eds) S. Garattini and E. B. Sigg. Excerpta Medical Foundation, Amsterdam, 1969.

35. Yen, C.Y., R.L. Stanger and N. Millman. Ataractic suppression of isolation induced aggressive behaviour. *Arch. Int. de Pharmacodyname.*, **123** : 179–185, 1959.