

in their emotionality are reported to differ in their benzodiazepine receptors (6). But association between emotionality and inter and intrasrain variations in the imipramine response on immobility time in forced swimming test (FST) is not reported in the literature. Hence in this study we aimed to separate Swiss and Balb/c strains of mice into different emotional groups based on their behavioral responses in novel arena and elevated plus maze and investigate the effect of imipramine on immobility time in FST in these emotionally different groups of mice.

METHODS AND MATERIAL

Inbred male mice of Swiss and Balb/c strain of 6–8 week old were used for the study. The animals were maintained on normal mice feed (Lipton feeds limited, Bangalore). All mice were maintained in clean cages at temperature ($21 \pm 1^\circ\text{C}$) and humidity ($55 \pm 2\%$) controlled, under 12 h reversed light cycle (lights off at 0700 hrs). Only experimentally naive animals were selected for the study. Clearance from the institutional ethical committee was obtained prior to starting the study and CPCSEA (Society for control and prevention of cruelty on animals headed by government of India) guidelines were followed throughout the study.

Separation of mice into groups on the basis of their emotionality

All mice were taken to the psychopharmacology lab and kept 1 h for acclimatization. Animals were evaluated for their indices of emotionality in novel arena and elevated plus maze.

Novel arena

This apparatus estimates animal's response to novelty and uncertainty. In this method both locomotor activity and emotionality are commonly estimated. The apparatus consists of a square wooden box (40X40X33 cm, LXBXH) with brown sunmica covering on all sides of inside walls and floor. The floor was partitioned into 16 small squares with distinct lines. A zero watt bulb was fixed on a glass top and observations were made through glass top. Each mouse was gently placed in the center of the Novel arena and direct visual observations were made through glass top for a period of 5 min. The apparatus was thoroughly wiped with ethanol after each trial. Indices of emotionality measured were number of line crossings and defecation score (7).

Elevated plus maze

The elevated plus-maze is constructed of sunmica, consists of two open arms (30X5 cm) and two enclosed arms (30X5X15 cm, LXBXH) arranged so that the arms of the same type were opposite to each other, connected by a central square (5X5 cm, LXB). From the centre four entries are there: two to closed arms and two to open arms. The maze is elevated to a height of 38.5 cm. Each mouse was placed at the intersection of the four arms of the elevated plus maze so that its head was facing towards the open arm of the platform. The indices of emotionality like number of entries to the open arms and time spent in the open arms are in the elevated plus maze was scored for 5 min under dim light (8). The apparatus was thoroughly wiped with ethanol/water (50% v/v) after each trial.

Method of separating mice into different emotional groups

Animals were segregated into normal emotional (NE), low emotional (LE), high emotional (HE), based on the performance of mice with respect to each indices of emotionality in novel arena and elevated plus maze. For example, segregation of mice into different emotional groups based on the number of line crossings in novel arena was done as described below: All mice which made line crossings between mean \pm 1SD value of the entire experimental subjects of each strain (100) were considered as NE, and mice which made line crossings more than mean + 1SD value were considered as LE and mice which made line crossings less than mean - 1SD value were considered as HE. With respect to defecation score, mice defecated less than mean - 1SD value were considered as LE and mice defecated more than mean + 1SD value were considered as HE. Only those mice that responded similarly for two indices of emotionality were segregated into respective emotional group and rest were rejected. A total of 100 mice of each strain were screened for the evaluation of emotionality in order to get 12 mice each of low and high emotional status.

Evaluation of influence of emotionality on imipramine response

Imipramine response was evaluated in NE, LE, HE mice of both Swiss and Balb/c strain by following forced swimming method (9). The apparatus consists of round container (45X20 cm, HXB) filled with water (25–27°C) up to a depth of 15 cm. The depth was deep enough so that mice could not

support themselves by placing their paws on the base of the cylinder used. Animals of each emotional group of strains were divided into control and test groups of six mice each and were taken for the study (Fig. 1). Mice of all emotional groups of both the strains were marked and weighed. The swim sessions were conducted by placing the NE, LE, HE mice of both the strains individually in glass cylinders and the basal immobility time (seconds) was recorded for 5 min duration. The water was changed between the subjects. Mouse was judged to be immobile when it is making only those movements necessary to keep its head above water. Next day these mice were given imipramine 20 mg/kg, po (Courtesy Micro laboratories Ltd) and swim sessions were again conducted after 30 minutes and immobility time in seconds in NE, HE, LE mice of both the strains was recorded (5).

Statistical analysis

ANOVA and posthoc Dunnet's 't' test were performed between different emotional groups of mice. Baseline immobility time and immobility time after imipramine administration of LE and HE mice were compared with that of NE mice using posthoc Dunnet's 't' test.

RESULTS

Separation of mice into groups on the basis of their emotionality

Mice of both the strains exhibited differences in their behavioral responses in both the models of emotionality. Based on

whether their number of line crossings and defecation score in novel arena (Table I) and number of entries to the open arms and time spent in open arms in elevated plus maze were below or above the mean (Table II) each strain of mice could be divided into normal emotional (NE), low emotional (LE) and high emotional (HE). Only those mice that responded similarly in both the models were considered to have the respective level of emotionality. Results of separation of mice into different emotional groups revealed that in Swiss mice strain 64 mice were found to be of normal emotional, 19 were high emotional and 17 were found to low emotional. Similarly in Balb/c mice strain 59 mice were found to be normal emotional, 22 mice were high emotional and 19 were low emotional.

Influence of emotionality on imipramine response

The results of comparison of baseline immobility time within the mice of a single strain revealed that the immobility time varied among different emotional groups of each strain. The baseline immobility time (seconds) was least with LE mice (Swiss: 68 ± 1.4 , Balb/c: 88.5 ± 2.1), intermediate with NE mice (Swiss: 83.75 ± 1.5 , Balb/c: 107.3 ± 2.2) and high with HE mice (Swiss: 104.1 ± 2.1 , Balb/c: 139 ± 1.7) in both the strains. ANOVA revealed significant differences in the baseline immobility time (seconds) among the different emotional groups of each strain (Swiss: $F_{(2,15)}: 144.43$, $P < 0.01$; Balb/c: $F_{(2,15)} = 152.31$; $P < 0.01$). Similar differences were observed in the immobility time (seconds) after imipramine administration in both the strain of mice.

TABLE I: Performance of Swiss and Balb/c mice in novel arena with respect to emotionality indices.

Strain	Emotionality indices in Novel Arena							
	Number of line crossings				Defecation score			
	Mean	SD	Mean+1SD	Mean-1SD	Mean	SD	Mean+1SD	Mean-1SD
Swiss	118.4	36.1	154.5	82.3	5.3	4.2	9.5	1.1
Balb/c	98.4	28.7	127.1	69.7	6.1	4.9	11.0	1.2

N = 100 per strain; SD : Standard deviation.

TABLE II: Performance of Swiss and Balb/c mice in elevated plus maze with respect to emotionality indices.

Strain	Emotionality indices in Elevated plus maze							
	Number of entries to open arms				Time spent in open arms (s)			
	Mean	SD	Mean+1SD	Mean-1SD	Mean	SD	Mean+1SD	Mean-1SD
Swiss	4.5	2.7	7.2	1.8	162.4	29.1	191.5	133.3
Balb/c	3.28	2.1	5.38	1.18	148.7	32.4	181.1	116.3

N = 100 per strain; SD : Standard deviation.

TABLE III: Effect of imipramine on immobility time in different emotional groups of Swiss and Balb/c mice.

Strain	Baseline immobility time (s)			Immobility time (s) after imipramine (20 mg/kg, po) administration		
	NE	LE	HE	NE	LE	HE
Swiss	83.75±1.5	68±1.4**	104.1±2.1**	22.2±0.7	20.3±0.3*	31.5±0.8**
Balb/c	107.3±2.2	88.5±2.1**	139±1.7**	20.8±0.8	13.87±1.1**	35.87±1.2**

N=6 each, NE: Normal emotional, LE: Low emotional, HE: High emotional; *P<0.05, **P<0.01 as compared to respective NE mice by posthoc Dunnet's 't' test.

Immobility time (seconds) after imipramine administration was least with LE mice (Swiss 20.3 ± 0.3 , Balb/c: 13.87 ± 1.1), intermediate with NE mice (Swiss mice 22.2 ± 0.7 , Balb/c $20.8 \pm .8$) and high with HE mice (Swiss 31.5 ± 0.8 , Balb/c 35.87 ± 1.2). ANOVA revealed significant differences in the immobility time (seconds) after imipramine administration in different emotional groups of both the strains (Swiss: $F_{(2,15)}; 210.34, P<0.001$; Balb/c: $F_{(2,15)} = 199.39; P<.001$). These results reveal that mice that differ in their baseline emotionality differ in their pharmacological response to imipramine (Table III).

DISCUSSION

It is well established that responses to drugs varies substantially across individuals, a phenomenon common to both humans and rodents. It is reported that there are species, strain and sex variations in emotionality and drug responses (2, 4, 5, 6). But the link between variations in emotionality within strains and its influence on drug responses is not reported in literature. In this study we attempted to segregate mice of Swiss and Balb/c strains into different emotional groups on the basis of their baseline emotionality. Balb/c mice

exhibited high emotionality when compared to Swiss mice as indicated by their response in novel arena and elevated plus maze (Table I, II). This may be due to lower density of benzodiazepine receptors in Balb/c mice as reported by Robertson (6). The response of mice with respect to the indices of emotionality in novel arena and elevated plus maze varied to a great extent (Table I, II) so that they could be divided into different emotional groups like normal emotional, low emotional and high emotional mice. Results of second experiment revealed the role of emotionality on inter and intra strain variations in baseline immobility time as well as the immobility time after imipramine administration. Comparison of baseline immobility time among different emotional groups of Swiss and Balb/c mice revealed that baseline immobility time is high with Balb/c mice when compared to Swiss mice. Comparison of baseline immobility time among NE, LE, HE groups of mice of each strain revealed that baseline immobility time was found to be more in high emotional groups of mice, intermediate in normal emotional groups of mice and least in low emotional groups of mice of both the strains. Similarly the effect of imipramine on the immobility time also differed among different emotional groups.

The immobility time in FST was least in LE groups of mice and intermediate with NE groups of mice and poor with HE groups of mice indicating that the response of imipramine was best in LE group, better in NE group and poor in HE group of mice. The high baseline immobility time in HE mice could be due to high depression. Stress is a known factor that increases anxiety in animals (10). There is a strong correlation between stress and depression. Low baseline immobility time in LE group of mice indicates their ability to counter any stressful situation. The results of our

experiments revealed the inter and intra strain variations in baseline emotionality in Swiss and Balb/c strains of mice and the influence of emotionality on pharmacological response of imipramine in forced swimming test.

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