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DIFFERENCES IN ACTIVE AVOIDANCE BEHAVIOUR OF HYPOACTIVE AND HYPERACTIVE RATS SUBJECTED TO IMMOBILISATION STRESS

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Abstract : Open field activity was studied in Wistar rats. Animals with low scores of ambulatory and rearing behaviours were grouped as hypoactive and those with high scores as hyperactive. Acquisition of active avoidance learning in a shuttle box was studied in the two groups. Hyperactive rats in contrast to hypoactive rats showed a better acquisition of avoidance learning. Learning was suppressed in both groups by domperidone, but was facilitated by immobilisation stress in the hypoactive group only. The two groups did not differ in the basal and stress evoked heart rates. These observations suggest that immobilisation stress favours enhancement of the dopaminergic related behaviour like avoidance learning in hypoactive rats.

Key words : immobilisation stress avoidance learning hypoactive rats hyperactive rats dopamine

INTRODUCTION

Based on individual differences in a specific behaviour, rats and other species have been categorised into hypoactive and hyperactive groups (1–5). Success in breeding two genetic strains lend support to genetic aetiology in respect of differences in active avoidance learning (6). These genetic rat lines showing absence or high level of active avoidance performance exhibit correspondingly low or high activity in open field (5, 7–12). But other patterns of behaviour like grooming or defaecation have not shown definite correlation to high or low performances in either a shuttle box or open field (2, 3, 13 13–17).

Neuronal and neurochemical mechanisms underlying these physiological differences in behaviour are not yet clearly defined. However, various observations suggest that this difference in activity may be associated with manifestation of varying degrees of autonomic (12, 18) and hormonal responses in basal and stressful situations (2, 3, 8, 10, 15, 19, 20–22).

Exposure to stress is reported to aggravate hypoactive behaviour in open

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field (23) indicating that these animals respond behaviourally in similar way under such stressful situations. On the other hand, as compared to Roman Low Avoidance rats, the Roman High Avoidance rats showed attenuated corticosterone response in open field and this difference between the two rat lines disappeared after immobilisation stress (IS) (8). Contradictory reports (2, 11, 12) indicate that the corticosterone response to stress is variable in the two types of rats.

It is, however, not clear whether the rats with low and high performances in open field and shuttle box will also continue to maintain this variation in avoidance learning after exposure to stress.

This study is designed to assess the effect of immobilisation stress on avoidance learning in two groups of rats selected on the basis of their activity in open field and shuttle box. The learning behaviour is further evaluated for identifying the possible role of dopamine in these activities in relation to immobilisation stress.

METHODS

Male Wistar rats aged 90 days (150–170 gm) were housed 5–6/cage in plastic cages (40 × 28 × 15 cm). All rats were derived from a breeding colony at our Institute and maintained at ambient temperature ($27^0 \pm 2^0$) under natural light dark cycles approximately 12/12 L.D. (lights on at 6.00 h) with free access to rat chow (Hindustan Lever, India) and water.

EXPERIMENTAL PROCEDURES

A. Open field behaviour

Open field behaviour (OFB) was studied in an open field chamber (2) measuring 100 × 100 × 40 cm with all its surfaces painted white. The floor was divided into 20 × 20 cm squares. OFB was recorded between 8.00 and 11.00 hours by keeping the chamber in a quiet room lit by two fluorescent lamps (40 W each) located 3.6 m above the centre of the field.

On the day of testing, the rat was placed in the corner of the chamber by a trained handler. The animal's behaviour was monitored by one of us (AC) for a period of 3 min to record (a) ambulation (the number of times the animal entered a square with all its four legs), (b) rearing, (c) grooming and (d) the number of defaecated boli.

The chamber was cleaned with 1% acetic acid after testing every animal.

B. Avoidance learning (AL)

A shuttle box (TKK Apparatus, Takaai and Co., Japan) comprising of two compartments each of 40 × 20 × 20 cm dimensions was used for avoidance learning. A guillotine door separated the left black compartment from the other painted white. Rats were allowed to explore the shuttle box for a period of min for two days preceding the learning session.

After placing the rat in one compartment of the chamber, learning trial commenced with shining of light (CS) with the help of 40 W bulb for 3 sec followed immediately

by an electric shock (UCS) (0.2 mA) given to the grid floor for a maximum period of 10 sec. Stimulus parameters were kept constant with the help of a stimulus controller connected to the shuttle box. Intertrial interval was 60 sec.

Each learning session comprised of 6 blocks of 10 trials each. Every rat thus underwent a maximum of 60 trials and the session lasted for 75–80 min between 9.00 to 12.00 hrs.

A trial was considered successful when the animal escapes to the other compartment with CS alone. Unsuccessful trials were classified as escape behaviour (EB) (crossing over during UCS) and freezing (FR) (failure to escape throughout the duration of UCS) AL, EB and FR were recorded during each block of 10 trials. Percentage of successful trials in the last block was taken as the maximum attainment of learning in that session. However, failure to attain even 20% success in a session was considered as the criterion for complete suppression of learning.

C. Immobilisation stress (IS)

Immobilisation stress (IS) was achieved by wrapping the rat on to a plexiglass board (24.5 × 21.4 cm; 350 g) (4) with cotton gauze passed through two slits on two sides of the board. The limbs and tail were fixed with adhesive tape. The rat was then placed supine with the board resting on it. IS was continued of a duration of 20 min.

D. Administration of domperidone (DMP) or vehicle

DMP (Janssen Pharmaceuticals, Newzealand) was diluted in a solution of ethanol (40%) and saline (60%) to a concentration of 12 mg/ml (24–26). DMP (4.0 mg/kg) or identical volume of diluting fluid (vehicle) was administered subcutaneously.

A. Open field behaviour (OFB)

Experiment No.1: A total of 103 rats were screened in the open field chamber for their OFB. Depending on the OFB scores, 29 rats were assigned to hypoactive group (HP) (less than 20 squares) and 40 rats to hyperactive group (HPR) (n = 40) (more than 40 squares). Rats with intermediate scores were excluded for other experimental protocols.

B. Avoidance learning (AL)

For experiments No. 2, 3 and 4 given below equal number [5] of rats were randomly selected from the hypo and hyperactive groups. Rats were not tested again after they had undergone training in shuttle box.

Experiment No.2: The purpose of experiment to analyse of performance of hypo– and hyperactive rats in the shuttle box.

Experiment No.3: In this experiment, each rat from hypo- and hyperactive group was injected with vehicle and 60 min later subjected to 20 min immobilisation stress which was followed by learning session in the shuttle box.

Experiment No.4: (a) In this protocol, each hypo- and hyperactive animal was tested for avoidance learning 60 min after DMP administration.

(b) Another set of hypo- and hyperactive animals were administered DMP and 60 min later each rat was subjected to 20 min immobilisation stress followed immediately by avoidance learning.

(c) Heart rate of all rat subjects to immobilisation stress procedure in experiments 3, 4(a) and 4(b) were monitored with ECG recordings (Physiograph, INCO, India).

Experiment No.5: Under anaesthesia (25% urethane in distilled water in a dose of 125 mg/100 gm intraperitoneally) hypoactive (n = 9) and hyperactive (n = 7) animals were placed in supine position and fixed to the dissection board. Through an incision in the neck, the right carotid artery was exposed and a polythene catheter filled with heparinised saline was introduced. It was connected to Statham Transducer P23 DC. BP was recorded on Grass Model 7 Polygraph, calibrated at 6 mm Hg/mm and speed 25 mm/sec. Heart rate was also counted from the BP recording.

The adrenal glands, both from hypo- and hyperactive groups of rats, (n = 7 each), were removed and weighed using electronic balance (DHONA, India) with reliability of 0.1 mg.

Statistical analysis

Open field data were subjected to ANOVA and paired `t` test. The strenght of association between ambulation and other behaviours in the open field was estimated by Pearson Product Moment Correlation coefficient. ANOVA was also used to compare AL between various hypo- and hyperactive rats subjected to shuttle box performance. Values are given mean ± SEM.

RESULTS

Experiment No. 1

Differentiation and selection of hypo- and hyperactive groups on the basis of ambulatory scores in open field (HP = 9.86 ± 6.7; HPR = 46.75 ± 6.3) was confirmed by statistical treatment of the data [F (1, 38) = 249.62; P<0.0001].

Rearing was significantly more in hyperactive group (P<0.001) (Table I). In the same group the defaecation scores were also higher (P<0.05) than in hypoactive group. Grooming behaviour was however similar (Table I).

Analysis of behaviour in open field showed that rearing (r = 0.68; P<0.001) and defaecation (r = 0.38; P<0.01) were highly correlated with ambulation. But no correlation was found between ambulation and grooming (r = 0.881).

Experiment No. 2

Higher attainment of active avoidance marked the shuttle box performance of hyperactive rats in comparison with that of hypoactive group (Fig.1). This difference in learning between the two groups was highly significant [F (1, 8) = 39.52; P<0.002].

Experiment No. 3

Exposure to 20 min of stress enhanced shuttle box performance in hypoactive rats as shown by increase in the number of

TABLE I : Open field behaviour scores for 3 min in hypoactive (HP) and hyperactive groups (HPR).

Group	*n*	*Ambulation*	*Rearing*	*Defaecation (No.of boli)*	*Grooming*
HP	29	9.86 ± 1.24 (0 – 20)	5.31 ± 0.81 (0 – 15)	2.50 ± 0.38 (0 – 8)	0.97 ± 0.24 (0 – 5)
HPR	40	46.75 ± 1.0 (40 – 64)	16.92 ± 1.01** (7 – 32)	4.18 ± 0.38* (0 – 9)	1.35 ± 0.22 (0 – 4)

Mean ± SEM; Figures in parentheses show range of activity
*P<0.05; **P<0.001.

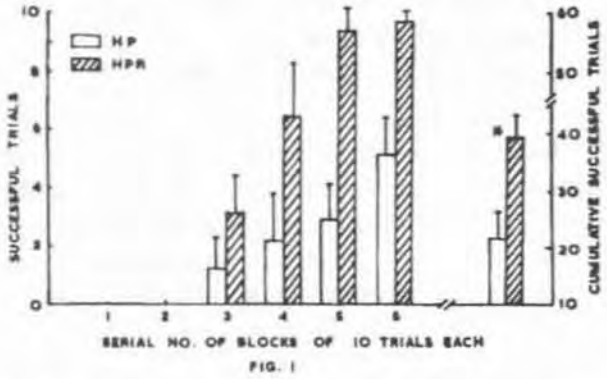

Fig.1: Acquisition of active avoidance learning in hypoactive (HP) rats (n=5) and hyperactive (HPR) rats (n=5). Cumulative responses of 60 trials are shown on the right side (*P<0.002).

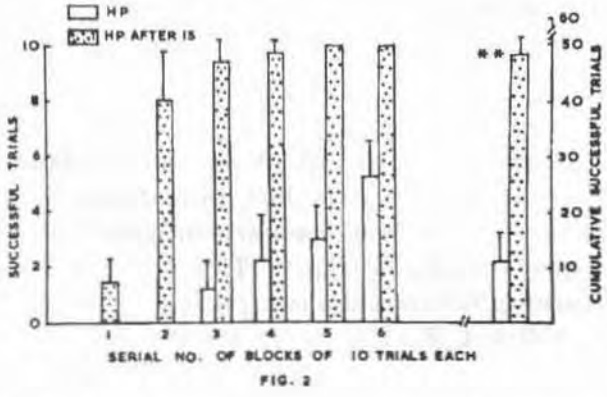

Fig.2: Acquisition of active avoidance learning in hypoactive (HP) control rats (n=5) and HP rats subjected to immobilisation stress (IS) (n=5). Cumulative responses are shown on the right side (**P<0.001).

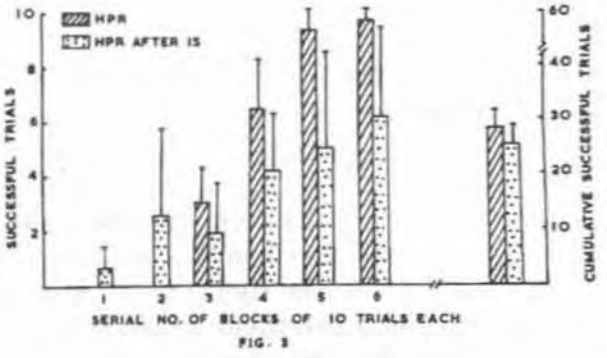

Fig.3: Acquisition of active avoidance learning in hyperactive (HPR) control rats (n=5) and HPR rats subjected to immobilisation stress (IS) (n=5). Cumulative responses are shown on the right side.

successful trials in initial blocks of training and earlier attainment of maximum learning at the end of 30 trials only (Fig.2) [$F(1,8) = 213.2$; $P<0.001$]. Stress did not alter this performance in the hyperactive group [$F(1,8) = 1.73$; $P<0.225$] (Fig.3).

Experiment No. 4

(a) Prior administration of DMP alone prevented acquisition of learning in hypo as well as hyperactive groups. DMP suppressed the learning to the same extent in both groups. Avoidance learning was replaced predominantly by freezing behaviour (Table II).

TABLE II : Shuttle box performance of hypoactive (HP) and hyperactive (HPR) rats after domperidone (DMP) treatment without and with stress (IS).

Group	*Experimental*	*Cumulative response in 60 trials*		
		Freezing	*Escape*	*Avoidance*
HP	DMP (n=5)	35.0 ± 6.38*	22.2 ± 5.62*	3.0 ± 1.27
	DMP and IS (n=5)	7.4 ± 4.13	50.2 ± 4.42	2.4 ± 6.78
HPR	DMP (n=5)	35.4 ± 6.77*	20.2 ± 7.98*	3.2 ± 1.24
	DMP and IS (n=5)	6.0 ± 3.15	49.4 ± 3.92	4.6 ± 1.36

Mean ± SEM: *P<0.01.

(b) Exposure of DMP treated rats to 20 min stress modified performance of hypo- and hyperactive rats in the shuttle box. Predominance of freezing behaviour seen after DMP treatment alone, was found to decrease substantially in DMP treated, stress exposed rats. Escapes were also enhanced in all rats after stress, but learning was completely suppressed, as none of the rats could attain two successful trials. Cumulative responses in Table II depicts the difference in these parameters.

Table III shows that the changes in heart rates during immobilisation stress in vehicle and DMP treated hypo- and hyperactive rats are similar.

Experiment No.5

Basal heart rates of hypo- and hyperactive rats showed no significant difference. Basal blood pressure was higher in hypoactive rats, but adrenal glands were heavier in hyperactive group (Table IV).

TABLE III : Heart rate per minute during immobilisation stress (IS) in hypoactive (HP) and hyperactive (HPR) rats with domperidone (DMP) treatment.

Groups	*Experimental*		*Minutes after IS*			
			0	*2*	*10*	*20*
HP	V and IS	(n=5)	332.0 ± 9.63	436.0 ± 16.44	447.2 ± 8.97	441.6 ± 9.93
	DMP and IS	(n=5)	339.2 ± 21.2	435.6 ± 11.07	444.0 ± 12.65	445.6 ± 16.23
HPR	V and IS	(n=5)	313.6 ± 8.06	426.4 ± 13.16	418.4 ± 19.78	429.6 ± 17.19
	DMP and IS	(n=5)	345.6 ± 23.65	430.4 ± 17.15	432.8 ± 14.05	426.8 ± 15.28

Mean ± SEM; V = Vehicle.

TABLE IV : Heart rate, blood pressure and adrenal gland weight in hypoactive and hyperactive rats.

Group	*Heart rat per min*	*Mean blood pressure mm Hg*	*Adrenal gland weight mg/100 g bw*
Hypoactive	335.6 ± 11.27	123.77 ± 4.74*	13.36 ± 5.28*
Hyperactive	324.6 ± 14.43	105.14 ± 6.97	15.314 ± 0.58

Mean ± SEM: *P<0.05

DISCUSSION

Selection of animals as hypoactive and hyperactive groups was based on extreme scores of activity in the open field (1, 16, 27). We found that 3% of the rats were immobile in open field. In an earlier study (2), higher incidence of immobility was reported. Species difference or inadequate sample size may have resulted in such disparity.

Efforts to correlate the level of emotionality in rats with rearing (1, 2, 8), grooming (2, 28, 29) and defaecation (2, 13, 15, 16, 28–30) have led to conflicting interpretations. Only rearing in this study was directly correlated with exploratory activity in the open field, indicating that there may be common neural mechanisms linking these behaviours, grooming is reported (8, 29) to be disturbed independently.

Rats demonstrating hyperactivity in open field acquire avoidance learning quicker than hypoactive rats. This confirms earlier observation (3, 5, 30, 31). Better performance may be owing to higher dopaminergic tone in hyperactive animals (15, 32–35).

The difference in shuttle box performance exhibited by hypo- and hyperactive rats disappear after exposure to immobilisation stress. Variability in hormonal response in Roman high and low avoidance rats was also absent after immobilisation stress (8). It is possible that stress enhances the sensitivity of dopaminergic mechanisms in hypoactive rats. Suppression of avoidance behaviour by dopamine receptor blocking is overcome to a certain extent after immobilisation stress (unpublished observation). These reports favour the view that central dopaminergic tone is an important determinant of behaviour in open field and shuttle box.

Domperidone, a central D_2 receptor blocking drug (24–26) suppressed shuttle box performance in all rats, but it did not alter basal or stress evoked heart rate response in hypo- and hyperactive rats. This precludes any peripheral blocking action of domperidone. However, stress probably augments central dopaminergic activity as revealed by some improvement in shuttle box performance by stress overcoming the domperidone effect. It is also likely that the dose of domperidone used did not abolish completely the dopaminergic activity. Increasing doses of domperidone will be helpful to clarify this mechanism.

From the available data in this study, it is not possible to explain higher blood pressure in hypoactive rats and heavier adrenals in hyperactive ones. It is suggested that use of peripheral sympathetic blockers and estimation of catecholamines and corticosterone levels may help to explain the underlying mechanism for these changes.

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