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SUBORDINATION INDUCED DECREASE IN 5-HYDROXYTRYPTAMINE AND DOPAMINE LEVELS IN THE FRONTAL CORTEX – A STUDY USING WORKER-PARASITE RELATIONSHIP IN RATS AS A MODEL

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Abstract : Competition for a limited resource appears to be an important factor in natural selection. Such competition when elicited experimentally, leads to the establishment of dominant–subordinate (D-S) relationship between the competitors. The present study was carried out to analyse the effect of D-S relationship on the levels of monoamines, namely, dopamine (DA), 5-hydroxytryptamine (5-HT) and norepinephrine (NE) in various brain regions. The model of D-S relationship selected for this work was a modified worker-parasite paradigm in adult male Wistar rats. The levels of monoamines were estimated in the frontal cortex, the entorhinal cortex, the hippocampus and the septum of the two competitors and a non-competitor control, using high pressure liquid chromatography (HPLC). Levels of DA and 5-HT, but not NE, were found to be lower ($P<0.05$) only in the frontal cortex of the subordinate as compared to that of the dominant or the control. These findings are comparable with similar neurochemical changes reported to be caused by some of the known stressors.

Key words : worker-parasite food-competition stomach contents dopamine 5-hydroxytryptamine frontal cortex

INTRODUCTION

Dominant–subordinate (D-S) relationship can be elicited in rats and other animals using various water/food–competition paradigms (1, 2, 3). Serotonergic and dopaminergic systems of the brain are reported to be mainly involved in this complex social behaviour. Prolonged subordination experience leads to an increase in the levels of 5-hydroxyindoleacetic acid (5-HIAA), the main metabolite of 5-hydroxytryptamine (5-HT) (4, 5) and a reduction in the amount of tryptophan, the aminoacid precursor of 5-HT (5) in many brain regions. However, the 5-HT levels are reported to be unchanged in the brain regions studied. Similarly, dopamine (DA) levels have been demonstrated to be unaltered by D-S relationship (4), although increasing the levels of dopamine in the brain promotes winning in the competitive interactions (6, 7).

The frontal cortex which shows changes in 5-HT and DA levels in response to stress situations (8, 9) has not been investigated with respect to D-S relationship. The septum which has profuse connections with the hippocampus and is involved in various learning and memory tasks (10) has also not been studied. Accordingly, the present work was aimed to study the effect of D-S relationship on the levels of 5-HT, DA and also norepinephrine (NE) in the frontal

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cortex and the septum in addition to some of the previously studied brain regions, namely, the hippocampus and the entorhinal cortex. The model of dominanace-subordination selected for this work was a worker-parasite relationship which can be established in a pair of rats exposed to food-competition (7). When two animals are competing for the reward, the competitor which ultimately gets more amount of reward is considered to be dominant whereas the other one is designated as subordinate (1, 3). We have used this criterion to determine the dominant or subordinate status of the animals in the worker-parasite paradigm.

METHODS

Animals:

Male Wistar rats (175-225 g) at the age of 80-90 days were grouped into triplets, each consisting of three weight-matched littermates. Littermates were taken to minimize genetic variation which could influence the parameters under study. All rats were individually housed in polypropylene cages (25 cm x 13 cm x 16 cm). One animal from each triplet was randomly assigned to be a non-competitor control and the other two were color coded and taken as competitors. Light-dark cycle was maintained at 12:12 hours schedule. Water was provided *ad libitum*.

Apparatus and procedure:

In the previous worker-parasite paradigm (7), two competitor animals are individually trained in an operant chamber to press a lever to get a reward. The trained animals are then paired and tested simultaneously in the operant chamber with a single lever and a single source of reward. The animal which makes at least 80% of the responses is termed as worker whereas the one which makes less than 20% of the responses is referred to as parasite. We have modified this model slightly by introducing two separate levers and keeping a single source of reward for the two competitors. In this modified paradigm the rats were not required to compete for pressing the lever but the competition was exclusively for the food. Accordingly, this is a better model for the food-competition behaviour than the previous one.

The operant chamber (Campden, UK) with dimensions of 20 cm x 23 cm x 24 cm, contained two levers (right and left) present on the same wall of the chamber with the food hopper in between them. The food could be accessed by one rat at a time. Lever responses were measured by an attached electronic counter. One of the two competitors was trained for the right and the other one for the left lever whereas the non-competitor control was trained for any one of the two levers. The animals were trained by continuous reinforcement (CRF) schedule. The shaping period started with placing the starved rats individually in the operant chamber where the reward (a food pellet of about 40 mg) was given by the experimenter whenever the animals approached the assigned lever. During this period which lasted for 3-5 sessions, animals learnt to press the assigned lever to get a food pellet. More training sessions were given to obtain a stable lever response rate (at least 10 responses/min). After the training sessions the rats were subjected to a test-period of 15 sessions when both the competitors were put together in the chamber and allowed to compete whereas the non-competitor control was tested individually. The number of lever responses and the time spent by each animal at the food hopper were noted in each session. The sessions were of 10-min duration, performed once in a day. Each animal was provided 5 g of additional food by the experimenter after the test-session.

Measurement of stomach contents:

The amount of food ingested by the animals during the test-sessions was assessed by measuring the amount of stomach contents immediately after the test-period, a method used previously (11). No additional food was given after the last test-session immediately after which the rats were sacrificed, their stomach contents scooped out and weighed. Starving a rat for 24 hours empties its stomach completely (unpublished observation) and hence the amount of stomach contents shown here represents the amount of food obtained by the rats during the last test-session.

Biochemical estimation:

The brains were rapidly removed on an inverted ice-filled Petri dish. Four brain regions, namely, the frontal cortex, the septum, the hippocampus and the entorhinal cortex were dissected out by a procedure based on a previous method (12). Levels of 5-HT, DA and NE were estimated using high pressure liquid chromatography (HPLC) coupled to a fluorescence detector as described elsewhere (13). Briefly, the HPLC system included a delivery pump (Erma, Japan), a reverse phase analytical column (Ultracarb 3 μm ODS 150 x 4.6 mm, Phenomenex, USA) which was protected by a guard column (Ultracarb, 3 μm ODS 30 x 4.6 mm, Phenomenex, USA), a degasser (Erma, Japan) and a fluorescence detector (Hitachi, Japan). The mobile phase (pH 3.92) consisted of sodium acetate (0.02 M), methanol (16% V/V), heptane sulfonic acid (0.1375% W/V) and EDTA (0.1 mM). Brain regions were homogenised in ice-cold 0.1 M perchloric acid (PCA) and centrifuged at 14,000 rpm for 15 min. The supernatant was filtered through a 0.45 μm membrane (Sartorius, Germany). A 100 μl aliquot of the filtrate was injected into the column and run at a flow rate of 0.9 ml/min. Isoproterenol was used as the internal standard. Excitation and emission wavelengths were fixed at 280 nm and 315 nm respectively. All separations were isocratic and the peaks for the three monoamines, namely, 5-HT, DA and NE were identified by comparison with standards. Calculations were done using the correction factor for the recovery of the internal standard.

Statistical analysis:

Lever response rates for the last training and all the 15 test sessions were analysed by two-way ANOVA (Treatment x Time x Interaction) with repeated measures on one factor (time). Significant results were further analysed by Newman-Keul's multiple comparisons tests. Weights of stomach contents were compared using one way ANOVA followed by Scheffe's multiple comparisons test. Values of monoamine levels were analysed by one-way ANOVA, followed by Newman-Keul's test for comparisons among the three groups.

RESULTS

Worker and parasite were defined on the basis of lever response rate of an animal and the time it spent at the food source. The animal emerging with higher level response rate but accessing the food source for less than 3 min in a 10 min session was called worker whereas the other one, by default, was termed as parasite. The dominant or subordinate status of an animal was decided on the basis of the amount of food ingested during the competition (as inferred from the amount of stomach contents measured after the competition). The competitor having ingested more amount of food was considered dominant and the other one, subordinate.

Lever response rate:

During the competition, animals rarely pressed the lever not assigned to them. Moreover, once established, the worker-parasite relationship was observed to be constant without any reversal. Statistical analysis of the lever response rates showed a significant effect of the treatment ($F_{2, 15}$=163.89; $P<<0.001$), the time ($F_{15, 225}$=59.71; $P<<0.001$) as well as their interaction ($F_{30, 225}$=76.65; $P<<0.001$). Further, the response rate of the control animal remained statistically unaltered throughout the test period while that of the two competitors decreased significantly ($P<0.01$) in the first test-session itself. This decline was progressive in the case of the parasite whereas the worker increased its rate after the initial decrease, till it reached a level comparable to that of the control (Fig. 1). Consequently, the response rate of the worker (and the control) became more than 30 times that of the parasite ($P<<0.001$), during the last test-session.

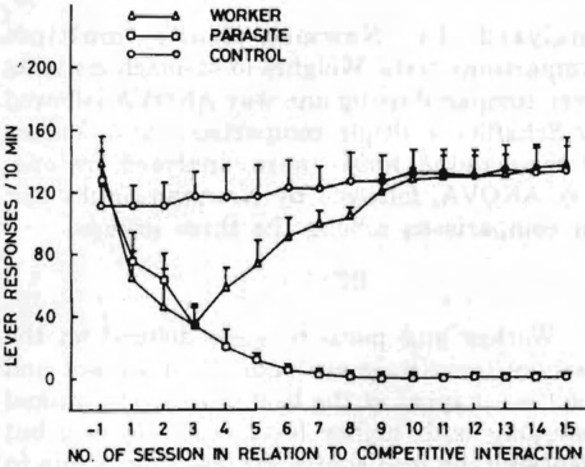

Fig. 1 : Lever response rates (Mean ± S.D.) of three groups of rats (n=6), recorded for the last training and all 15 test-sessions. Lever response rate of the worker was significantly ($P<<0.001$) higher than the parasite but not the control.

Amount of stomach contents:

Analysis of the stomach contents data revealed differences among the groups ($F_{2, 15}=5.25$; $P<0.05$). Pairwise comparisons showed that the amount of stomach contents was significantly less in the case of the worker as compared to the parasite (S=3.84; $P<0.05$) or the control (S=4.03; $P<0.05$), whereas there was no difference between the parasite and the control (Fig. 2). Accordingly, the worker behaved as a subordinate and the parasite as a dominant animal during the competitive interactions.

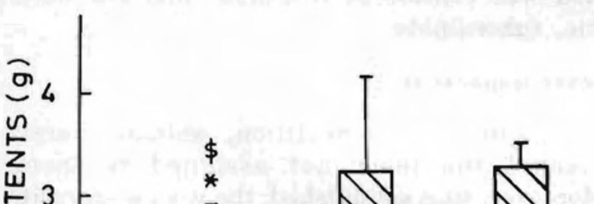

Fig. 2 : Amount of stomach contents (Mean ± S.D.) obtained from three groups of animals (n=6) immediately after the last test session which was performed at least 24 hours after the preceeding session. The amount of stomach contents was significantly ($P<0.05$) lower in the case of the worker as compared to the parasite (*) or the control ($).

Levels of monoamines:

There was a significant reduction in the levels of DA ($F_{2, 15}=4.01$; $P<0.05$; Fig. 3) and 5-HT ($F_{2, 15}=5.16$; $P<0.05$; Fig. 4) in the frontal cortex of the subordinate when compared to that of the dominant (27% and 22% reduction in DA and 5-HT respectively) or the control (23% and 25% reduction in DA and 5-HT respectively). No significant difference was found in DA or 5-HT levels in the hippocampus, septum or entorhinal cortex, among the three groups. Similarly, there was no significant alteration in the levels of NE in any of the brain regions analysed ($P>0.05$, Fig. 5).

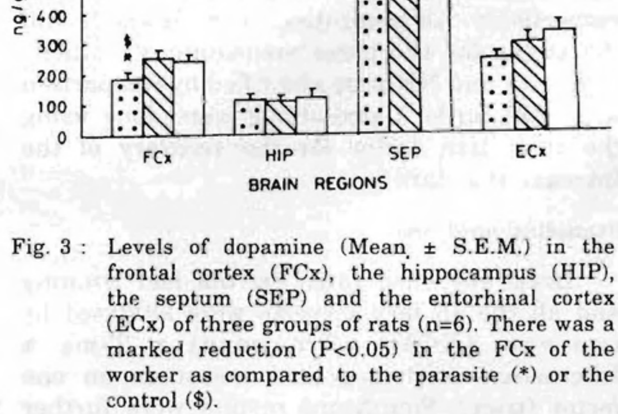

Fig. 3 : Levels of dopamine (Mean ± S.E.M.) in the frontal cortex (FCx), the hippocampus (HIP), the septum (SEP) and the entorhinal cortex (ECx) of three groups of rats (n=6). There was a marked reduction ($P<0.05$) in the FCx of the worker as compared to the parasite (*) or the control ($).

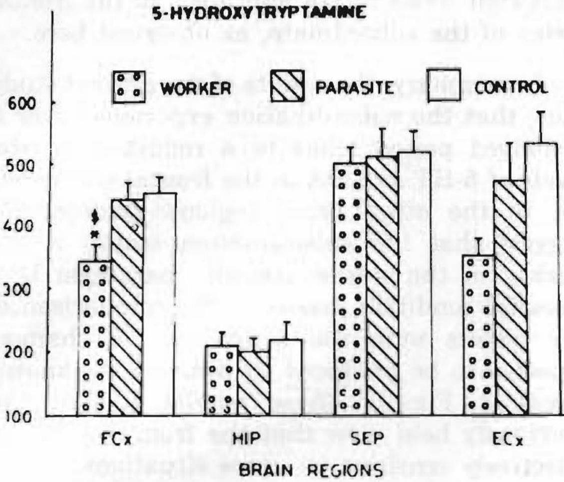

Fig. 4 : Levels of 5-hydroxytryptamine (Mean ± S.E.M.) in the frontal cortex (FCx), the hippocampus (HIP), the septum (SEP) and the entorhinal cortex (ECx) of three groups of animals (n=6). There was a significant reduction ($P<0.05$) in the FCx of the worker as compared to the parasite (*) or the control ($).

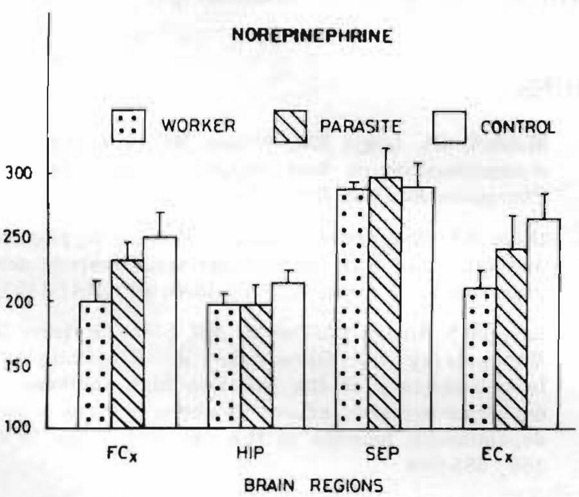

Fig. 5 : Levels of norepinephrine (Mean ± S.E.M.) in the frontal cortex (FCx), the hippocampus (HIP), the septum (SEP) and the entorhinal cortex (ECx) of three groups of animals (n=6). There was no statistical difference among various groups.

DISCUSSION

The worker-parasite paradigm was selected as the model of D-S relationship in this study for three reasons: (i) this is similar to food or water-competition models previously used to determine the dominant or subordinate status of the animals (1, 3); (ii) this type of model is considered to be more objective, quantifiable and advantageous than some other models based on more or less phenomenological observations (1); (iii) the food-competition behaviour appears to be an important factor for natural selection (14) and success in such competition could be important for the individual survival (2).

The most important finding in the present study was a significant decrease in the levels of DA and 5-HT occurring only in the frontal cortex of the subordinate as compared to that of the dominant or the non-competitor control. These changes could possibly be due to the less food consumption by the subordinate animal. However, provided that 5 gm of additional food was given to all the animals after testing, it appears unlikely that these changes are caused by less food intake by the subordinates. The only other factor is the subordinate status which might be responsible for these changes. Unaltered levels of 5-HT in the hippocampus and the entorhinal cortex observed in this study is in agreement with a previous report (4). Similarly, the septum also did not show any change. Reduced levels of 5-HT and DA occurring only in the frontal cortex, may indicate that the frontal cortex is selectively vulnerable to the insult caused by subordination. Further, the subordinate status may act as a stressful condition as some of the stressors are known to produce similar neurochemical changes in the frontal cortex. Inescapable foot-shock for example, decreases the 5-HT levels in the frontal cortex of mice (8, 15, 16). Similarly, foot-shock of various duration leads to decreased DA levels in the frontal cortex of rodents (9, 17). Cold restraint stress also alters the dopaminergic turnover in the frontal cortex (18).

It is intriguing why the frontal cortex alone shows such changes in response to stress. Mesocortical dopaminergic neurons may have higher selective sensitivity to stress situations (9, 19), possibly due to the lack of autoreceptors in the terminals of the mesocortico-frontal DA neurons (18, 20). The present finding of decreased levels of DA and 5-HT, occurring only in the frontal cortex, may suggest that there is some kind of interaction between dopaminergic and serotonergic neurons terminating in the frontal cortex. Chemical and electrolytic lesioning of the serotonergic neurons have been shown to alter the basal discharge rate of certain dopaminergic neurons (21). Furthermore, 5-HT neurons innervate the dopaminergic neurons in the ventral tegmental area (VTA) which may influence the mesocortical system (22). In fact, the raphe neurons, particularly those originating from the median raphe, have a facilitatory control on the activity of the mesocortico-frontal neurons (23). It is possible that the subordination stress of the worker leads to a reduction of the 5-HT levels in the serotonergic neurons projecting to the frontal cortex as well as those to the VTA; the latter inturn causing a reduction of the DA levels in the mesocortico-frontal neurons, ultimately resulting in the decreased levels of DA and 5-HT in the frontal cortex of the subordinate, as observed here.

In summary, the results of the present study show that the subordination experience over a prolonged period leads to a reduction in the levels of 5-HT and DA in the frontal cortex but not in the other brain regions studied. We suggest that the subordination status of the worker in the worker-parasite paradigm is a stressful condition, based on the comparison of our results with the neurochemical changes reported to be produced by some of the known stressors. Finally, these results support the previously held view that the frontal cortex is selectively sensitive to stress situations.

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